NOVEMBER 10, 1941

Lewis

THE JOURNAL OF NUTRITION

VOLUME 22 NUMBER 5

GEORGE R. COWGILL, *Editor*
Yale University School of Medicine, New Haven, Conn.

HAROLD H. MITCHELL, *Associate Editor*
University of Illinois, Urbana, Ill.

EDITORIAL BOARD

ALBERT G. HOGAN — ICIE MACY-HOOBLER — HOWARD B. LEWIS
ERNEST B. FORBES — HENRY A. MATTILL — ALFRED T. SHOHL
PHILIP A. SHAFFER — LELA E. BOOHER — GEORGE O. BURR
CONRAD A. ELVEHJEM — RUSSELL M. WILDER

Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA, PA.

Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign

Entered as second-class matter January 20, 1934, at the post office at Philadelphia, Pa., under Act of March 3, 1879. Copyright 1941, The Wistar Institute of Anatomy and Biology. All rights reserved.

Publications of The Wistar Institute

JOURNAL OF MORPHOLOGY

Devoted to the publication of original research on animal morphology and physiology, including cytology, protozoology, and the embryology of vertebrates and invertebrates. Articles do not usually exceed 50 pages in length.

Issued bimonthly, 2 vols. annually: $20.00 Domestic, $21.00 Foreign, per year.

THE JOURNAL OF COMPARATIVE NEUROLOGY

Publishes the result of original investigations on the comparative anatomy and physiology of the nervous system.

Issued bimonthly, 2 vols. annually: $15.00 Domestic, $16.00 Foreign, per year.

THE AMERICAN JOURNAL OF ANATOMY

Devoted primarily to the interests of anatomy as taught in the medical schools of the United States and to the development of scientific anatomy in these schools. Publishes researches only.

Issued bimonthly, 2 vols. annually: $15.00 Domestic, $16.00 Foreign, per year.

THE ANATOMICAL RECORD

Organ of the American Association of Anatomists and the American Society of Zoologists

For the prompt publication of concise original articles on vertebrate anatomy, preliminary reports; technical notes; critical notes of interest to anatomists and short reviews of noteworthy publications.

Issued monthly, 3 vols. annually: $22.50 Domestic, $24.00 Foreign, per year.

THE JOURNAL OF EXPERIMENTAL ZOOLOGY

Publishes papers embodying the results of original researches of an experimental or analytical nature in the field of zoology.

Issued 9 times a year, 3 vols. annually: $22.50 Domestic, $24.00 Foreign, per year.

AMERICAN JOURNAL OF PHYSICAL ANTHROPOLOGY

Organ of the American Association of Physical Anthropologists

In this journal appear original papers on anthropology in general; researches in evolution, man's origin; human ontogeny; variations in man; heredity; eugenics; demography; the American Indian and abnormal classes. Papers on anthropometry and the methods of anthropology also appear.

Issued quarterly, 1 vol. annually: $6.00 Domestic, $6.50 Foreign, per year.

JOURNAL OF CELLULAR AND COMPARATIVE PHYSIOLOGY

Publishes papers which embody the results of original research of a quantitative or analytical nature in general and comparative physiology, including both their physical and chemical aspects.

Issued bimonthly, 2 vols. annually: $10.00 Domestic, $11.00 Foreign, per year.

THE JOURNAL OF NUTRITION

Organ of the American Institute of Nutrition

Publishes original researches in the field of nutrition and occasional reviews of literature on topics with which the journal is concerned.

Issued monthly, 2 vols. annually: $10.00 Domestic, $11.00 Foreign, per year.

THE AMERICAN ANATOMICAL MEMOIRS

Publishes original monographs based on experimental or descriptive investigations in the field of anatomy which are too extensive to appear in the current periodicals. Each number contains only one monograph. List of monographs already published, with prices, sent on application.

ADVANCE ABSTRACT CARD SERVICE

Every paper accepted for publication in one of the above periodicals is accompanied by the author's abstract. The abstract and the complete bibliographic reference to the paper as it will eventually appear is printed on the face of a standard library catalogue card. This Advance Abstract Card Service is issued promptly, in advance of the journal containing the paper, and is offered in three styles.

Prices per year $2.00, $2.50 and $3.00, postpaid.

These publications enjoy the largest circulation of any similar journals published.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

Woodland Avenue and Thirty-sixth Street, Philadelphia, Pa.

DIFFERENCES IN THE BEHAVIOR OF RATS AND MICE TOWARDS DEFICIENCIES OF CERTAIN MEMBERS OF THE VITAMIN B COMPLEX [1]

JOHN R. FOY AND LEOPOLD R. CERECEDO
Department of Chemistry, Fordham University, New York

FIVE FIGURES

(Received for publication June 18, 1941)

At least two well-recognized types of dermatitis have been observed in rats fed on diets deficient in different fractions of the vitamin B complex. One of these is the specific dermatitis or acrodynia due to vitamin B_6 deficiency and the other is the non-specific dermatitis due to pantothenic acid deficiency. In the present work we have sought to duplicate these skin manifestations in mice.

On diets usually employed to produce acrodynia in the rat, that is, a vitamin B complex-free diet supplemented with thiamine and riboflavin, mice show only a non-specific dermatitis which does not respond to vitamin B_6 but is completely cured by the further addition of pantothenic acid. Uncomplicated vitamin B_6 deficiency can be produced in both rats and mice by supplementing a vitamin B complex-free diet with thiamine, riboflavin, and some source of the "filtrate factor" freed from vitamin B_6. However, the characteristic acrodynia seen in rats under these conditions does not appear in mice, not even on low-fat diets.[2]

[1] Presented at the meeting of the American Chemical Society at St. Louis, April, 1941.

[2] The reduction in the amount of fat in the diet minimizes the possible curative effect of the unsaturated fatty acids on the acrodynia and emphasizes the difference between the symptoms in rats and mice.

THE JOURNAL OF NUTRITION, VOL. 22, NO. 5
NOVEMBER, 1941
Copyright 1941
The Wistar Institute of Anatomy and Biology.
All rights reserved.

EXPERIMENTAL

In the experiments with mice, weanling albino animals from a strain reared in our laboratory were used in all cases. They were placed on the basal diets at 21 to 25 days of age, at which time they weighed from 8 to 12 gm. In the rat experiments, animals of the Wistar strain were used. They were also 21 to 25 days of age and weighed 35 to 40 gm. at weaning. The percentage composition of the diets used is given in table 1.

TABLE 1

Percentage composition of the diets.[1]

CONSTITUENTS	DIET			
	A	B	C	D
"Vitamin-free" casein [2]	31	30		
Ether-extracted casein			30	20
Sucrose	38	55	63	75
Osborne and Mendel salt mixture	7	7	7	5
Hydrogenated cottonseed oil [3]	21	5		
Cod liver oil	3	3		

[1] Vitamin supplements were used as indicated in the text.
[2] Labco.
[3] Crisco.

Diets C and D were supplemented with concentrates of vitamins A and D.[3] The concentrates were dissolved in ether and evaporated on casein so that at least 6000 I.U. of vitamin A and 600 I.U. of vitamin D were present in each 100-gm. portion of the diet. When used for feeding and injections the water soluble vitamins (thiamine,[4] riboflavin,[4] nicotinic acid,[4] pyridoxine,[5] and pantothenic acid [6]) were dissolved in 0.1 N HCl and kept in the icebox. The solutions were neutralized before use. When they were included in the diet an aqueous solution of the vitamins was evaporated on a small

[3] Generously supplied by Dr. I. Harris.
[4] Obtained from Merck and Co., Rahway, N. J.
[5] First made available to us through the courtesy of Dr. Samuel Lepkovsky and later obtained from Merck and Co.
[6] Generously supplied by Merck and Co.

amount of casein which was then thoroughly mixed with the other constituents.

Experiments on mice receiving the vitamin B complex-free diet plus limited supplements of the crystalline factors

After the discovery of vitamin B_6 deficiency in the rat had been reported by György ('34, '35), it was decided to repeat this work on mice under similar conditions. The experiments were carried out on five groups of animals. The results are presented in figure 1, the legend of which gives the pertinent details.

Groups 1 to 4 inclusive (fig. 1) were fed diet A. Since a high fat content of the diet is known to exert a sparing action on certain factors of the vitamin B complex (Birch and

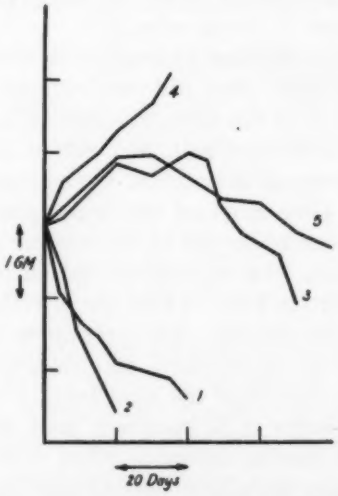

Fig. 1 Average growth curves of five groups of mice kept on a vitamin B complex-free diet supplemented with various factors of the vitamin B complex. Groups 1, 2, 3 and 4 received diet A and daily doses of the following supplements: Group 1 (twenty-one animals), 2 μg. thiamine and 5 μg. riboflavin (curve 1); group 2 (nine animals), 2 μg. thiamine (curve 2); group 3 (eight mice), 4 μg. thiamine, 5 μg. riboflavin and 10 μg. nicotinic acid (curve 3); group 4 (eight animals), 4 μg. thiamine and 10 μg. riboflavin (curve 4). Group 5 (forty mice) received diet B plus 5 μg. thiamine and 10 μg. riboflavin per day (curve 5).

György, '36; Stirn et al., '39), and since unsaturated rats in particular show a curative action on vitamin B_6 deficiency (Salmon, '40; Schneider et al., '40), the high-fat (21%) ration (diet A) was abandoned in favor of the low-fat (5%) mixture (diet B). Group 5, consisting of forty mice, received diet B supplemented with 5 μg. of thiamine and 10 μg. of riboflavin. After a slight initial growth these animals lost weight and died within 80 days (curve 5). As in the previous experiments, the symptoms associated with vitamin B_6 deficiency in the rat were not evident, but certain non-specific symptoms were observed. That these mice were not suffering primarily from vitamin B_6 deficiency is shown by the fact that the administration of as much as 20 μg. of pyridoxine daily for 10 to 20 days to each of ten animals after the dermatitis had developed was entirely without effect either on the symptoms or on the growth of the mice.

The following symptoms were seen to a greater or lesser degree in all animals that did not receive supplements of pantothenic acid or of the filtrate factor. The mice developed a humped back, awkward gait, and yellow matted fur which became thin, falling out in patches. Occasionally wet brownish scales appeared over most of the body, and in a few cases ulcerous sores were observed in the region of the axilla and groin. The animals were extremely emaciated, the skin being drawn tightly over the body so that the injection of the vitamin solution was made difficult. The extremely sensitive and irritable condition of the animals manifested itself in a characteristic kicking twitch of the hind legs.

From these results it is apparent that small amounts of thiamine and riboflavin, adequate when supplementing more complete diets, are definitely subminimal on the incomplete diets, and that under conditions in which the requirement for the vitamin B complex is only partially met, an increased amount of fat in the diet gives the animals a better chance for growth and health than the low-fat diets. Although these experiments indicate a difference between rats and mice, the diet used with mice was somewhat higher in protein and salts

than that commonly employed in rat experiments. It was therefore decided to observe the effect on the growth and symptoms of both rats and mice kept on the same diet when one vitamin B complex factor at a time was added to the basal ration.

The influence of certain factors of the vitamin B complex on the growth and symptoms of rats and mice maintained on identical vitamin B complex-free rations

Two groups of forty-eight mice and eighteen rats were each divided equally into six groups and given diet B with the supplements listed in table 2.

TABLE 2

Supplements furnished to the rats and mice in comparative studies on these two species.

GROUP	SUPPLEMENTS (IN MICROGRAMS PER DAY)							
	Thiamine chloride		Riboflavin		Pyridoxine		Nicotinic acid	
	Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats
1								
2	5	20						
3	5	20	10	40				
4	5	20	10	40	20	20		
5	5	20	10	40			20	40
6	5	20	10	40	20	20	20	40

The crystalline vitamins were dissolved in 0.1 cc. of water and injected into the mice but fed to the rats. The growth curves for both the rats and the mice are given in figures 2 and 3, printed side by side to facilitate comparison.

Group 1. These animals received only the basal diet. Both the mice and rats lost weight and developed a humped back and an emaciated, unkempt appearance, but no specific symptoms were evident (curve 1).

Group 2. In this experiment diet B was supplemented with thiamine. The mice, after a slight initial growth, lost weight until death, while the rats just about maintained their weight.

Since the animals lived longer, the symptoms were more fully developed, but the characteristic acrodynia was not evident in either case (curve 2).

Group 3. In these animals which were given diet B plus thiamine and riboflavin, growth was better than that of the two preceding groups (curve 3). The symptoms observed in the mice were those that have already been described. The rats, however, developed symptoms characteristic of vitamin

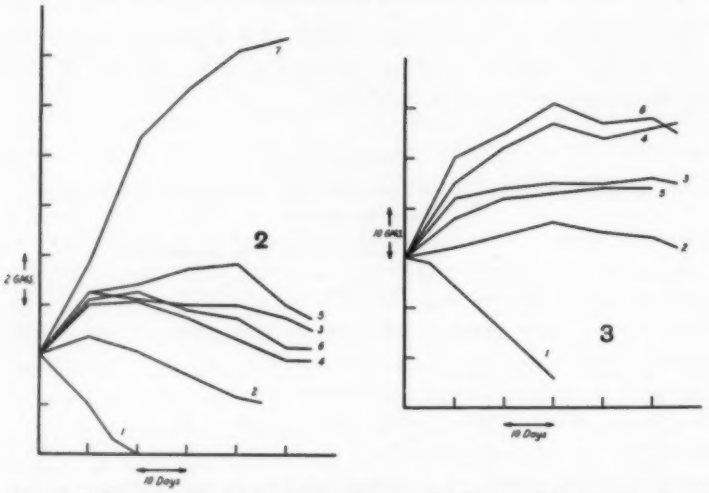

Fig. 2 Effect of various crystalline factors on the growth response of mice maintained on a vitamin B complex-free ration (diet B). Group 1 received only diet B (curve 1). The other six groups were given diet B and the following supplements (see table 2): Group 2, thiamine (curve 2); group 3, thiamine and riboflavin (curve 3); group 4, thiamine, riboflavin and pyridoxine (curve 4); group 5, thiamine, riboflavin and nicotinic acid (curve 5); group 6, thiamine, riboflavin, pyridoxine and nicotinic acid (curve 6); group 7, thiamine, riboflavin, pyridoxine and pantothenic acid. The animals in group 7 were not litter mates of the other mice in this experiment but the large difference in the growth rate is significant. In experiments in which the growth rates are similar small differences are significant only if litter mates are used in each group. Thus curve 7 cannot be compared with the normal growth shown in curve 4 of figure 4.

Fig. 3 Effect of various crystalline factors on the growth response of rats maintained on diet B. Group 1 received only diet B (curve 1). The other five groups (curves 2–6) received the same supplements which were given to the corresponding groups in the mouse experiment (fig. 2).

B_6 deficiency, that is, swollen paws and ears with cracking and denuding of the skin of the paws and muzzle.

It would appear from these results that on the same diet, mice do not develop the same symptoms as rats. Whereas the rat develops the characteristic acrodynia, the mouse develops a non-specific dermatitis of the type usually associated with filtrate factor deficiency in the rat.

Group 4. In this experiment the animals were given diet B plus thiamine, riboflavin, and pyridoxine. The growth of the mice did not differ significantly from that obtained with thiamine and riboflavin alone, while the rats showed a definite growth response to the added vitamin B_6 (curve 4). Except that the usual symptoms were preceded by an alopecia and a dry dandruff-like dermatitis, the syndrome in the case of the mice was that previously described. The rats, on the other hand, were free from the acrodynia seen in group 3, and developed instead the non-specific dermatitis attributed to filtrate factor deficiency and now known to be cured by pantothenic acid.

Groups 5 and 6. Those animals receiving nicotinic acid in addition to the other factors differed little in growth and symptoms from animals in the corresponding groups that did not receive this factor (curves 5 and 6).

For comparison we include the growth curve of a group of mice receiving diet B plus thiamine, riboflavin, pyridoxine, and pantothenic acid (Sandza and Cerecedo, '41). These animals grew at a rate that was about 80% of normal and were free from all symptoms (curve 7).

If we compare the results obtained with these two species, the most striking difference is the lack of response to vitamin B_6 and the similarity of the symptoms in all groups of mice, while in the rats two distinct syndromes are evident, and there is a definite growth response to each added factor except nicotinic acid. In the mouse, after the primary deficiency of thiamine and riboflavin has been satisfied, vitamin B_6 in the absence of pantothenic acid has little or no effect on growth or symptoms. A similar lack of response to concentrates of

factor W (prepared according to Black et al., '40) has been observed in mice subsisting on a diet containing only thiamine, riboflavin, and pyridoxine.[7] That the symptoms observed in the mice receiving these incomplete diets were caused by the absence of pantothenic acid is evident from the experiment of Sandza and Cerecedo ('41). A number of workers have reported this factor to be responsible for the cure of the non-specific dermatitis in rats.

Attempts to produce uncomplicated vitamin B_6 deficiency in the mouse using liver filtrate as a source of the filtrate factor

The necessity of feeding mice pantothenic acid or some source of the filtrate factor in order to produce uncomplicated vitamin B_6 deficiency, or to observe the effect of supplements

TABLE 3

Diets and supplements furnished to the mice and rats in the study of vitamin B_6 deficiency.

GROUP	NO. OF		DIET	DAILY SUPPLEMENTS							
				Thiamine chloride		Riboflavin		Pyridoxine		Liver filtrate	
				γ		γ		γ		cc.	
	Mice	Rats		Mice	Rats	Mice	Rats	Mice	Rats	Mice	Rats
1	3	3	B								
2	18	15	B	5	10	10	20			0.15	0.3
3	6	10	B	5	10	10	20	5	10	0.15	0.3
4	3	20	Stock[1]								

[1] The stock diet given to the mice had the following composition: Ground oats (Quick Quaker or Sunbeam) 50 parts, whole milk powder (Klim, or Merrell-Soule) 15 parts, and dried yeast (Northwestern) 10 parts. The rats received Purina Dog Chow as stock diet.

of pyridoxine, is evident from the above experiments. Consequently, a fuller's earth filtrate of autolyzed pig liver was prepared by the method of Lepkovsky and his co-workers ('36). Each cubic centimeter of this liver filtrate was equivalent to 10 gm. of whole liver. This experiment was conducted on four groups of mice and rats maintained on the diets and

[7] Unpublished data.

supplements listed in table 3. The growth curves for these animals are shown in figures 4 and 5.

Group 1. These animals received only diet B. The results with both the rats and the mice were the same as those that have been reported above (curve 1).

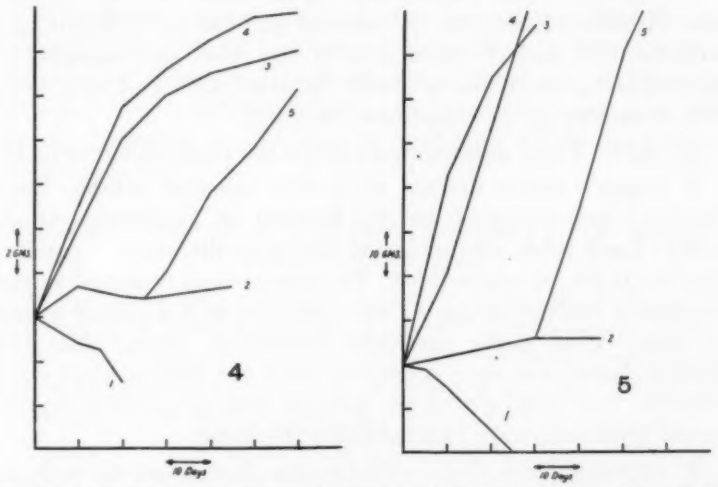

Fig. 4 Average growth curves of mice. In this experiment a source of the filtrate factor was given. Group 1 represents the negative controls (three animals) which were given only diet B (curve 1). Groups 2 and 3 received diet B and the following supplements: Group 2 (eighteen mice), thiamine, riboflavin and liver filtrate (curve 2); group 3 (six mice), thiamine, riboflavin, pyridoxine and liver filtrate (curve 3). Group 4 (three animals), was kept on the stock diet (curve 4). Some of the mice in group 2 received pyridoxine in addition to the other supplements (curve 5).

Fig. 5 Average growth curves of rats. This experiment is a duplicate of that conducted on mice and shown in figure 4. The number of animals in each group is given in table 3.

Group 2. The animals in this group received diet B plus thiamine, riboflavin and the liver filtrate. Both species maintained their weight within certain narrow limits (curve 2). No specific syndrome was evident in the mice though they became weak and emaciated, developed a humped back and walked with an awkward gait. In a few cases the eyes were

closed by a serous exudate. The rats developed the acrodynia characteristic of vitamin B_6 deficiency except for the absence of any pronounced dermatitis of the ears. Both the mice and the rats responded well to doses of vitamin B_6 in growth and cure of symptoms (curve 5).

Group 3. In this experiment the animals received diet B plus thiamine, riboflavin, pyridoxine and the liver filtrate. In both the rats and the mice growth was good as compared to the normal growth obtained with the stock diet, and both were free from any gross symptoms (curve 3).

Group 4. These animals were given the stock diet (curve 4).

A fuller's earth filtrate of a 92% alcohol soluble liver extract,[8] was prepared by the method of Lepkovsky et al. ('36). Each cubic centimeter of this liver filtrate was equivalent to 10 gm. of whole liver. The experiment reported in the foregoing section (group 3) was repeated with a second group of thirty mice under the same conditions, except that the filtrate factor was that prepared from the 92% alcohol liver extract. The results with the mice as well as parallel experiments with rats were substantially the same.

It appears from these experiments that mice, as well as rats, require vitamin B_6 for normal nutrition, but that the symptoms of a deficiency of this factor do not express themselves in the same manner in each species, at least under the conditions of the present work.

Mice have been kept on diet B supplemented with thiamine, riboflavin, pyridoxine, and the liver filtrate for periods of 100 days. Though the growth was not quite normal, the animals weighed 85% as much as their litter mate controls kept on the stock diet, and showed no gross symptoms at any time. This is not in agreement with the findings of Norris and Hauschildt ('40). On a diet not qualitatively different from our diet B, they observed failure of growth and skin lesions in their mice similar to the dermatitis which we observed in animals given only thiamine, riboflavin, and pyridoxine. However, in our experiments an equivalent of 1.5 gm. of liver was

[8] Supplied by the Wilson Laboratories, Chicago.

given per day while Norris and Hauschildt gave an equivalent of only 0.5 gm. every other day. It has also been our experience that some preparations of liver filtrate do not furnish the activity expected, either in rats or mice. Norris and Hauschildt do not report that they checked their results on rats, using the same source of liver filtrate.

In a recent report Martin ('41) has shown that the deficiency described by Norris and Hauschildt is due to pantothenic acid. This is a confirmation of both our experiments in which the liver filtrate furnished the pantothenic acid, and those of Sandza and Cerecedo ('41) in which pantothenic acid was fed as such.

Attempts to produce vitamin B_6 deficiency in the mouse, using a low-fat diet

Since it had been shown that the dermatitis associated with vitamin B_6 deficiency may be cured by the presence of certain fatty acids in the diet, an attempt was made to produce the characteristic acrodynia in mice on a low-fat diet. These experiments were conducted on two groups of rats and mice.

Group 1. Forty mice were given diet C (table 1) supplemented with 2.5 mg. of thiamine, 5.0 mg. of riboflavin, and an equivalent of 750 gm. of liver as liver filtrate, per kilo of diet. Although the animals remained practically stationary in weight and became increasingly worse in appearance, no evidence of acrodynia was observed. The animals died before the symptoms of fatty acid deficiency could develop. Since the work of Burr and Burr ('29, '30) had been repeated on mice in this laboratory,[9] these symptoms were known to us.

Group 2. Twelve rats were given diet D (table 1) supplemented with 10 μg. of thiamine chloride, 20 μg. of riboflavin and an equivalent of 3 gm. of liver as liver filtrate per day. All animals developed the characteristic acrodynia of vitamin B_6 deficiency.

Thus it appears that even on low-fat diets mice, unlike rats, do not develop the specific dermatitis usually associated with a lack of vitamin B_6.

[9] Unpublished data.

SUMMARY

On vitamin B complex-free diets supplemented with thiamine and riboflavin, mice, unlike rats, do not develop a specific dermatitis nor do they exhibit any growth response to the addition of pyridoxine to their daily ration.

Mice fed a vitamin B complex-free diet develop a non-specific dermatitis in the presence of any or all of the known factors of the vitamin B complex (thiamine, riboflavin, pyridoxine, and nicotinic acid) with the exception of pantothenic acid.

On a vitamin B complex-free diet supplemented with thiamine, riboflavin, pyridoxine, and the filtrate factor, mice grow at a rate that is about 85% of normal and show no gross lesions of a deficiency of any kind over a period of 100 days.

LITERATURE CITED

Birch, T. W., and P. György 1936 A study of the chemical nature of vitamin B_6 and methods for its preparation in a concentrated state. Biochem. J., vol. 30, p. 304.

Black, S., D. V. Frost and C. A. Elvehjem 1940 The relation of vitamin B_6 and pantothenic acid to factor W studies. J. Biol. Chem., vol. 132, p. 65.

Burr, G. O., and M. M. Burr 1929 A new deficiency disease produced by rigid exclusion of fat from the diet. J. Biol. Chem., vol. 82, p. 345.

———— 1930 On the nature and rôle of the fatty acids essential in nutrition. J. Biol. Chem., vol. 86, p. 587.

György, P. 1934 Vitamin B_6 and the pellagra-like dermatitis in rats. Nature, vol. 133, p. 498.

———— 1935 Investigations on the vitamin B_2 complex: I. The differentiation of lactoflavin and the "rat anti-pellagra" factor. Biochem. J., vol. 29, p. 741.

Lepkovsky, S., T. H. Jukes and M. E. Krause 1936 The multiple nature of the third factor of the vitamin B complex. J. Biol. Chem., vol. 115, p. 557.

Martin, G. J. 1941 The mouse antialopecia factor. Science, vol. 93, p. 422.

Norris, E. R., and J. Hauschildt 1940 A new factor of the vitamin B complex required by the albino mouse. Science, vol. 92, p. 316.

Salmon, W. D. 1940 The supplementary relationship of vitamin B_6 and unsaturated fatty acids. J. Biol. Chem., vol. 133, p. lxxxiii.

Sandza, J. G., and L. R. Cerecedo 1941 Requirement of the mouse for pantothenic acid and for a new factor of the vitamin B complex. J. Nutrition, vol. 21, p. 609.

Schneider, H., H. Steenbock and B. R. Platz 1940 Essential fatty acids, vitamin B_6, and other factors in the cure of rat acrodynia. J. Biol. Chem., vol. 132, p. 539.

Stirn, F. E., A. Arnold and C. A. Elvehjem 1939 The relation of dietary fat to the thiamin requirements of growing rats. J. Nutrition, vol. 17, p. 485.

THE ASSIMILATION OF FLUORINE BY RATS FROM NATURAL AND SYNTHETIC CRYOLITE AND FROM CRYOLITE-SPRAYED FRUITS[1,2]

MARGARET LAWRENZ AND H. H. MITCHELL
Division of Animal Nutrition, University of Illinois, Urbana

(Received for publication June 30, 1941)

In preceding papers from this laboratory, the effects of various dietary factors and methods and forms of administration on the assimilation of fluorine by growing rats have been studied. This report will be concerned with the effects of factors more specifically related to the fluorine in spray residues on fruits.

Cryolite is the most important fluorine-containing insecticide employed in the spraying of fruits (Carter and Busbey, '39). There are two forms of cryolite on the market at the present time, one the natural mineral and the other a synthetic product. While the former is more widely used as an insecticide, especially in the Northwest, the latter has been the form used in our experiments up to the present. There are reasons for believing that the two commercial cryolites may possess different assimilabilities and toxicities. The natural product is a purer form of sodium fluoaluminate, Na_3AlF_6, than is the domestic synthetic product (Carter, '39), and according to our own analyses it contains more fluorine,

[1] This experiment was made possible by the donation of funds to the University of Illinois by the Aluminum Company of America and the Pennsylvania Salt Manufacturing Company.

[2] This investigation was conducted under the general supervision of a Committee on the Physiological Effects of Spray Chemicals, appointed by the Director of the Agricultural Experiment Station and consisting of the following members: H. H. Mitchell, W. A. Ruth, W. P. Flint and Julia P. Outhouse.

and its fluorine is somewhat less soluble. It seemed important, therefore, to compare the two products as to their assimilation by the growing rat.

The toxicity of cryolite for the insects infesting fruit against which it is effective is greater, within limits, the more finely the cryolite is ground. It seemed important to ascertain whether the fineness of division was a factor in determining the toxicity of cryolite for a higher form of animal life such as the rat.

During the weathering of sprayed apples and peaches on the tree, there is a possibility that the chemical composition of the spray residue may be changed, either through chemical reactions or by the differential leaching of more soluble constituents in the case of an impure chemical such as cryolite. These possible changes, although they have not, to the writers' knowledge, been investigated in the case of cryolite, may alter the assimilability of the contained fluorine. Hence, this possibility seemed worthy of study.

During the growth of the apple and during storage subsequent to harvesting, a waxy coating accumulates on the surface of the fruit (Markley and Sando, '31), the nature of which has been largely revealed by the investigations of Sando ('23, '31) and van der Haar ('24). The production of this wax coat apparently interferes with the commercial removal of spray residues from apples, and also its rate of formation may be stimulated by the application of sprays (Markley and Sando, '33). Therefore, it seemed expedient to study the possibility that waxing of the apple may impair the assimilation by animals of the fluorine component of the residue from cryolite-sprays.

PLAN OF THE EXPERIMENTS

The experiments were planned along the same general lines as those previously reported (for example, Lawrenz, Mitchell and Ruth, '39). In each of the four experiments to be reported, twelve pairs or trios of rats, depending upon the number of rations to be compared, were selected on the basis of sex,

litter membership and body weight, and were fed the experimental rations in equal amounts within the pair or trio. At the beginning of each experiment, samples of one to three or four rats were taken from each litter contributing rats to the experiment, for determination of the initial content of fluorine. After the consumption of a definite amount of food, generally 500 gm. or 1000 gm., unless unanticipated circumstances dictated otherwise in the case of individual pairs or trios, the experimental rats were sacrificed, their empty weights and body lengths determined, the carcasses separated into bones, teeth and soft tissues after autoclaving at 13 pounds pressure for 15 to 25 minutes depending upon the size of rat, and these three portions weighed and analyzed for fluorine by methods previously described (Lawrenz, Mitchell and Ruth, '39). During the feeding period, the rats were weighed weekly, and after the sixth week the teeth were examined weekly for the appearance of striations, using a jeweler's lens with a magnification of 4 ×.

All experimental rations contained low levels of fluorine, approximating 10 p.p.m., in order that the results obtained with them may possess more significance with reference to the prevailing fluorine hazard in the diet of the American people. They were all designed to contain all of the known essential nutrients in adequate amounts.

In the first experiment a comparison was made of the assimilation of fluorine from a domestic synthetic cryolite [3] and from a natural Greenland cryolite.[4] The former was very finely divided, its particles being mostly 1 μ or less in diameter. The latter was obtained in two forms; one, the commercial product, consisted of much larger particles (5 μ or less) than those in the synthetic cryolite; the other, a specially prepared product, was ground to a degree of fineness comparable to that of the synthetic product. The fluorine contents of these three cryolite preparations were 44.9% for the synthetic cryolite, 46.2% for the coarse natural cryolite and 50.5% for

[3] Provided by the Aluminum Company of America.
[4] Provided by the Pennsylvania Salt Manufacturing Company.

the special fine natural cryolite. The higher fluorine content of the finely ground as compared with the coarsely ground natural cryolite may have been due to the variation among samples of the mineral or to the rejection, in the process of grinding, of coarser material relatively poor in fluorine. These three cryolite samples were added separately to basal rations of natural foods (wheat, dry milk solids), supplemented with minerals and cod liver oil, to form three experimental rations containing 9.6 p.p.m. of fluorine of which 8 parts were derived from the added cryolites. A comparison of the assimilation of fluorine from the ration containing the synthetic cryolite and from that containing the fine natural cryolite was expected to throw light upon any differential toxicity of the two preparations due to differences in chemical composition. A comparison of the two rations containing the finely ground and the coarsely ground natural cryolite should reveal any appreciable differential toxicity due to differences in particle size.

The second experiment was planned to test the assimilation of fluorine by the rat from unweathered and weathered natural cryolite in one comparison, and from weathered coarse natural cryolite and weathered fine natural cryolite in another. Through the kindness of Professor W. P. Flint, arrangements were made to secure, from a commercial orchard near Carbondale, Illinois, Elberta peaches that had been sprayed (rather late in the season) with a spray mixture containing in one case the coarsely ground natural cryolite, and in the other, the finely ground special natural cryolite. In addition, unsprayed peaches were secured from the same source. Under the direction of Dr. W. A. Ruth, these peaches were dried at a low temperature (70° F.) and ground to a fine powder. The peach powders were found to contain the following amounts of fluorine: coarse cryolite sprayed, 100 p.p.m., fine cryolite sprayed, 94 p.p.m., and unsprayed, 1.5 p.p.m. The two sprayed-peach powders were incorporated into similar basal rations containing equal amounts of casein, starch, sugar, and corn oil, with mineral and vitamin additions, in such propor-

tions as to provide 8 p.p.m. of fluorine. This required 8.5% of the fine-cryolite sprayed peach powder and 8.07% of the coarse-cryolite sprayed peach powder. To the latter ration was added enough of the unsprayed peach powder (0.43%) to equalize the content of peach powder in the two rations. A third ration was prepared by adding to the same mixture of basic foods 8.5% of unsprayed peach powder and enough finely ground natural cryolite to provide 8 p.p.m. of fluorine. Thus, the three rations to be tested in this experiment contained equal amounts of all organic and mineral nutrients including peach powder, and equal amounts of added fluorine in the form of unweathered fine natural cryolite, weathered fine natural cryolite, and weathered coarse natural cryolite. The total fluorine content of all rations was 9.6 p.p.m.

The third experiment was similar to the second except that weathered synthetic cryolite on sprayed Ben Davis apples, dried and ground, containing 10 p.p.m. of fluorine, was compared with unweathered synthetic cryolite mixed with unsprayed apple powder, containing 1.05 p.p.m. of fluorine, prepared from Huntsman apples. Both rations contained 65% of apple powder, equal and adequate amounts of casein, minerals, vitamins and other organic nutrients, and equal proportions of fluorine, i.e., 6.8 p.p.m.

The fourth experiment was concerned with a possible effect of the formation of a wax coat on sprayed apples on the assimilation of fluorine in spray residues. For this purpose, Jonathan apples sprayed with synthetic cryolite were used. One lot of freshly picked apples, not over-ripe, was dried immediately at a low temperature and ground to a fine powder under the supervision of Dr. Ruth. Another lot of the same apples was allowed to stand at a fairly warm temperature for several weeks to "wax up." They were then dried and ground. The apple powders contained 32 and 30 p.p.m. of fluorine, respectively, and were incorporated in the usual basal balanced mixture of organic and mineral ingredients in amounts to provide about 11 p.p.m. of fluorine. The content of apple powder in both rations was equalized at 35%

by adding a small amount (2.3%) of unsprayed and unwaxed apple powder to the ration containing the apple powder (unwaxed and sprayed) with the larger content of fluorine. Both rations contained, by actual analysis, 13.2 p.p.m. of fluorine.

RESULTS OF THE EXPERIMENTS

The average results of the four experiments are summarized in table 1. In table 2 will be found a statistical analysis of the paired data secured in the first experiment, using the method of Student ('25).

In the first experiment, the growth of the rats was quite similar on the three experimental rations and was fairly rapid, averaging 1.65 gm. per day. Tooth striations made their appearance in the various rats within a period extending from the end of the eighth week to the end of the ninth week, with no perceptible differences between groups.

The fluorine retention data for the first two series of rats, receiving the synthetic and natural cryolite ground to a comparable degree of fineness, showed highly significant differences, with reference to the fluorine content of bones and teeth and the total fluorine retention. An average of 13.1% more fluorine was retained by the rats receiving synthetic cryolite than by their trio mates receiving natural cryolite and the probability that this, or a greater, difference would occur as the result of uncontrolled fortuitous factors is only 0.013. The percentage difference in bone fluorine is 12.4 and in tooth fluorine, 5.1. Although the fluorine in the soft tissues, expressed either as milligrams or parts per million, averaged higher for the rats receiving synthetic cryolite than for those receiving natural cryolite, the probability of a chance outcome was much too large to be disregarded.

It may be concluded, therefore, that the fluorine in synthetic cryolite is definitely and significantly more completely assimilable by the rat than the fluorine of natural cryolite. Furthermore, this difference cannot definitely be attributed to a difference in size of particles, since the two cryolites were

TABLE 1

The growth data and fluorine metabolism data secured in the four experimental comparisons. Each figure is an average for twelve rats.

	EXPERIMENT 1			EXPERIMENT 2			EXPERIMENT 3		EXPERIMENT 4	
	Averages for rats receiving:			Averages for rats receiving:			Averages for rats receiving:		Averages for rats receiving:	
	Synthetic cryolite	Fine natural cryolite	Coarse natural cryolite	Unweathered fine natural cryolite	Weathered fine natural cryolite	Weathered coarse natural cryolite	Weathered synthetic cryolite	Unweathered synthetic cryolite	Unwaxed apples	Waxed apples
Initial body weight, gm.	39	39	38	49	49	49	50	50	33	32
Total gain in weight, gm.	183	183	185	207	204	205	149	157	136	137
Final body length, mm.	221	221	221	227	225	225	205	207	204	204
Total food consumed, gm.	1000	1000	1000	1000	1000	1000	884	884	500	500
Numbers of food refusals	13	7	12	13	3.8	8.7	12	11	8	8
Total intake of fluorine, mg.	9.65	9.63	9.70	9.6	9.6	9.6	6.002	6.030	6.600	6.600
Feeding period, days	111	111	111	118	118	118	90	90	69	69
Final empty body weight, gm.	222	222	223	256	253	254	199	207	169	169
Weight of dry, fat-free bones, gm.	11.487	11.723	11.581	11.896	11.911	11.666	10.253	10.319	8.485	8.354
Weight of dry teeth, gm.	0.408	0.427	0.422	0.462	0.449	0.459	0.409	0.399	0.360	0.367
Weight of soft tissues, gm.	199.6	197.9	201.2	234	231	232	179	187	154	153
Fluorine in bones, p.p.m.	313	272	265	291	295	298	251	252	305	302
Fluorine in bones, mg.	3.565	3.170	3.051	3.437	3.501	3.454	2.537	2.563	2.578	2.519
Fluorine in teeth, p.p.m.	180	163	163	143	156	153	208	216	186	176
Fluorine in teeth, mg.	0.073	0.070	0.068	0.066	0.070	0.070	0.085	0.086	0.067	0.064
Fluorine in soft tissues, p.p.m.	0.39	0.36	0.40 [1]	0.34	0.39	0.38	0.73	0.89	0.55	0.56
Fluorine in soft tissues, mg.	0.079	0.072	0.080 [1]	0.072	0.082	0.082	0.128	0.173 [2]	0.084	0.084
Total fluorine in carcass, mg.	3.717	3.312	3.199 [1]	3.575	3.653	3.606	2.750	2.822	2.729	2.667
Initial fluorine content, mg.	0.230	0.228	0.227	0.318	0.317	0.316	0.317	0.320	0.214	0.209
Total fluorine retained, mg.	3.487	3.084	2.972 [1]	3.257	3.336	3.290	2.433	2.502	2.515	2.458
Total fluorine retained, per cent	36.14	32.03	30.70	33.92	34.75	34.27	41.83	42.47	38.12	37.26

[1] Because the soft tissue sample of one of the rats in this series was obviously contaminated with fluorine, these averages involve eleven instead of twelve rats.

[2] Omitting one sample (from a rat that received unweathered cryolite) with a suspiciously high F content, suggesting contamination with bone, this average becomes 0.141.

ground to comparable degrees of fineness. Determinations of the relative solubilities of the fluorine in the two cryolites offer a clue to their different physiological effects. Solubility tests over a limited period of time were made upon 5 mg. and 25 mg. samples of the two cryolites, using water, 0.2% HCl and 0.5% HCl as the solvents, the latter two solvents simulating the acidity in gastric contents and gastric juice, respectively. In both series of tests the fluorine in synthetic cryolite was

TABLE 2

Statistical analysis of fluorine data.

	SYNTHETIC VS. NATURAL CRYOLITE				FINELY GROUND VS. COARSELY GROUND NATURAL CRYOLITE			
	Mean difference[1]	Standard deviations of differences	t value	P	Mean difference[2]	Standard deviations of differences	t value	P
Fluorine in bones, p.p.m.	+40.9	22.1	6.14	<0.00007	+7.7	15.5	1.65	0.064
Fluorine in bones, mg.	+0.395	0.294	4.45	0.0005	+0.130	0.173	2.49	0.015
Fluorine in teeth, p.p.m.	+17.1	11.6	4.88	0.0002		insignificant		
Fluorine in teeth, mg.	+0.00358	0.00463	2.56	0.013		insignificant		
Fluorine in soft tissues, p.p.m.		insignificant				insignificant		
Fluorine in soft tissues, mg.		insignificant				insignificant		
Total fluorine retained, mg.	+0.403	0.296	4.51	0.0005	+0.117	0.173	2.24	0.025

[1] The positive sign indicates that the mean difference favors the synthetic cryolite.

[2] The positive sign indicates that the mean difference favors the finely ground natural cryolite.

more readily soluble in water than the fluorine in natural cryolite, the difference amounting to 10 to 11%. In dilute HCl, the tests with the 5 mg. samples revealed no consistent difference in solubility while the tests with the larger samples revealed a 10% difference.

Comparison of the second and third series of rats, receiving natural cryolite of two degrees of fineness, reveals a definite and significant difference in total fluorine retention and especially in the fluorine content of the bones, averaging about 4%. The greater fluorine retentions were secured with the

finer ground cryolite, although solubility tests failed to reveal any consistent differences in the solubility of fluorine in the two mineral preparations, either in water or dilute HCl.

The results of experiment 2, also summarized in table 1, reveal no significant differences among the three series of rats on application of Student's method of assessing the significance of paired differences, either in rate of gain, appearance of tooth striations, fluorine contents of tissues or total fluorine retention. Thus, the effect of weathering on the assimilability of fluorine from finely ground natural cryolite is inappreciable, and the effect of grinding, shown to be a real effect in the preceding experiment, is obliterated by weathering. This result is analogous to the finding of Shields, Mitchell and Ruth ('39) that the weathering of lead arsenate spray residue on apples does not appreciably modify the assimilability of the contained lead.

In the test of the effect of weathering on synthetic cryolite, the tooth striations characteristic of fluorosis appeared at the beginning of the tenth week of feeding, and in nine of the twelve pairs appeared first among the rats on the unweathered cryolite. Three rats on the weathered cryolite showed no striations at the end of 16 weeks of feeding, when they were sacrificed for analysis. Although the fluorine contents of the bones, teeth and soft tissues, and the total fluorine retentions, averaged higher for the rats that received unweathered cryolite than for their pair mates that received weathered cryolite, the differences between averages were quite insignificant, when tested statistically, except, anomalously, in the case of the soft tissues. Here, leaving out the one suspiciously high analysis noted in the table, the average difference was 0.0126 mg., the standard deviation of differences 0.0143 mg., and the probability of a fortuitous event only 0.0076. Although the data of the experiment are not equally convincing in respect to each of the items measured, it seems reasonable to conclude that, with synthetic cryolite in contrast to natural cryolite, weathering on the fruit will in some degree lower the assimi-

lability of its fluorine, conceivably by leaching out some or all of the more soluble compounds of fluorine contained in it.

The fourth experiment revealed no great difference between the assimilation of fluorine from waxed and unwaxed apples. Although the average retention of fluorine during the feeding period and the total content of fluorine in bones and teeth were somewhat smaller for the rats receiving the waxed-apple powder, the differences between pair mates in any one comparison were not consistently in the direction indicated by the respective averages. An application of the statistical method of analysis revealed such a high probability that the outcome may have resulted from chance that any conclusion that waxing was the determining factor, or even an appreciable factor, could not be defended.

Reference to table 1 will show that the concentration of fluorine in the teeth of the rats receiving waxed-apple powder averaged considerably lower than the concentration of fluorine in the teeth of the rats that received unwaxed-apple powder, 176 p.p.m. as compared with 186 p.p.m. The differences between pair mates favored the ration containing unwaxed apples in nine of the twelve pairs and averaged 10.7 p.p.m. With a standard deviation of differences of 13.9 p.p.m. the probability of a fortuitous result is only 0.0135, so that the conclusion that it was brought about by the difference in wax coating on the apples supplying most of the dietary fluorine can be defended strongly. However, the total fluorine contents of the teeth, when submitted to a similar analysis, yield a probability of 0.12, surely too large to be neglected. Tooth striations appeared indiscriminately among the two groups of rats beginning with the seventh week of feeding, so that these observations afford no reason for distinguishing between the two experimental rations with reference to fluorine toxicity. It is possible, however, that the method of preparing the apples for test, particularly the fineness of grinding, may have largely obliterated an appreciable effect of waxing on the assimilation of fluorine residues when the apples are consumed in the intact form.

SUMMARY AND CONCLUSIONS

Four experiments have been performed on growing rats, involving the controlled feeding and chemical analysis of twenty-four trios and twenty-four pairs of animals. These experiments were designed to detect and measure differences in retention by animals of the fluorine in natural Greenland and domestic synthetic cryolite and possible modification of such retentions by the particle size of mineral, by the weathering of spray chemicals on the fruit and by the development of the wax coating on the surface of sprayed apples.

The results obtained appear to warrant the following conclusions:

1. The fluorine of synthetic cryolite is appreciably more completely retained by animals than the fluorine of natural cryolite, probably a result of the greater solubility of the fluorine contained in it.

2. The degree of fineness to which cryolite is ground may modify the assimilation of the contained fluorine.

3. The weathering of natural Greenland cryolite on sprayed fruit does not appreciably affect its assimilability by animals nor presumably its toxicity.

4. On the other hand, with synthetic cryolite, weathering on the fruit will in some degree lower the assimilability of its fluorine, conceivably by leaching out some, or all, of the more soluble compounds of fluorine contained in it. Thus, the differences observed between synthetic and natural cryolite tested in an unweathered form will tend to disappear when the two cryolites are applied to fruit in spray mixtures and subjected to weather conditions.

5. Possibly the development of a wax coat on apples sprayed with cryolite impairs the assimilability of the contained fluorine, as it seems to render more difficult the removal of spray residue by commercial washing. The experiments reported in this paper relating to this subject, while not particularly assuring in support of this conclusion, quite probably did not permit the full effect of waxing to be exerted.

LITERATURE CITED

CARTER, R. H. 1939 Significant properties of some cryolite materials offered for insecticidal use. J. Econ. Ent., vol. 32, pp. 490–492.

CARTER, R. H., AND R. L. BUSBEY 1939 The use of fluorine compounds as insecticides, a review with annotated bibliography. U. S. Dept. Agr., Bureau of Ent. and Plant Quarantine, Div. of Insecticide Investigations, Washington, D.C., February, 1939, pp. 145.

HAAR, A. W. VAN DER 1924 Untersuchungen über der Saponine und verwandte Körper. XIII. Die Identität von Malol mit Urson (Ursolsäure). Rec. Trav. Chim. Pays-Bas., vol. 43, pp. 548–549.

LAWRENZ, M., H. H. MITCHELL AND W. A. RUTH 1939 The comparative toxicity of fluorine in calcium fluoride and in cryolite. J. Nutrition, vol. 18, pp. 115–125.

MARKLEY, K. S., AND C. E. SANDO 1931 Progressive changes in the waxlike coating on the surface of the apple during growth and storage. J. Agr. Res., vol. 42, pp. 705–722.

———— 1933 Possible changes in the waxlike coating of apples caused by certain spray and other treatments. Plant Physiol., vol. 8, pp. 475–478.

SANDO, C. E. 1923 Constituents of the wax-like coating on the surface of the apple. J. Biol. Chem., vol. 56, pp. 457–468.

———— 1931 Ursolic acid. J. Biol. Chem., vol. 90, pp. 477–495.

SHIELDS, J. B., H. H. MITCHELL AND W. A. RUTH 1939 The effect of apple constituents on the retention by growing rats of lead contained in spray residues. J. Nutrition, vol. 18, pp. 87–97.

"STUDENT" 1925 New tables for testing the significance of observations. Metron, vol. 5, pp. 105–120.

α-TOCOPHEROL REQUIREMENT OF THE RAT FOR REPRODUCTION IN THE FEMALE AND PREVENTION OF MUSCULAR DYSTROPHY IN THE YOUNG

MARIANNE GOETTSCH AND ALWIN M. PAPPENHEIMER

Departments of Biochemistry and Pathology, College of Physicians and Surgeons, Columbia University, New York City

(Received for publication June 30, 1941)

Vitamin E is essential for normal reproduction in the female rat (Evans and Bishop, '23; Mattill and Stone, '23; Sure, '23–'24) and for the prevention of muscular dystrophy in the young (Evans and Burr, '28; Olcott, '38; Goettsch and Ritzmann, '39). Biological methods for estimating the vitamin are but roughly quantitative (Palmer, '37; Bacharach, '38), and relatively little has been recorded upon the α-tocopherol requirement of the rat (Evans, Emerson and Emerson, '36; Karrer and Keller, '38; Bacharach, '38; Mason, '40).

This is a report on the potencies of natural α-tocopherol, synthetic *dl*-α-tocopherol, and synthetic *dl*-α-tocopherol acetate with respect to:

A. Cure of sterility in the female rat. (1) Production of litter: (a) size; (b) number of living young. (2) Number of second and third litters. (3) Success of lactation: (a) number of young surviving 10 days; (b) incidence of muscular dystrophy.

B. Prevention of muscular dystrophy in the young rat.

EXPERIMENTAL

Diets and supplements

The diets (I and II), similar to those used by Evans and Burr ('27), had the following percentage composition:[1] casein[2] 29.1

[1] The figures for diet I are those which immediately follow the name of the ingredient; the figures for diet II are given in parentheses.

[2] Merck's technical.

(26.6); cornstarch, raw 36.3 (33.3); lard 20.0 (18.3); yeast, bakers' dried 9.0 (16.6); salt mixture (Hawk and Oser, '31) 3.6 (3.4); and cod liver oil[3] 2.0 (1.8). Diet II containing more yeast was used during the period of lactation. The diets were freshly prepared every week.

Three preparations of α-tocopherol were employed: synthetic *dl*-α-tocopherol acetate[4] and natural and synthetic α-tocopherols.[5] The tocopherols were dissolved in ethyl laurate or sesame oil. The solutions were prepared by weight in such proportions that for feeding, 0.5 mg. of α-tocopherol was contained in 1 drop, and for injection the desired amount was present in 0.1 cc. of sesame oil. The solutions were stored at 4°C. and used within 1 week of preparation.

Methods

Repeated tests were carried out with small groups of animals over a period of 2 years. This tended to cancel out variations due to unrecognized differences in food composition, possible seasonal influences, or differences in individual response.

Young female rats, which during lactation had been on a natural-foods diet containing small amounts of vitamin E (Mason and Bryan, '38), were reared on diet I from the time of weaning (21 days) and bred with normal males at 90 days (Evans and Bishop, '23; Palmer, '37). It was held unnecessary to prove the sterility of the rats by a preliminary resorption gestation, as in previous experiments forty-six females reared and bred on diet I had failed to cast a single litter.

Single doses of α-tocopherol were given orally or parenterally on or before the fifth day following the finding of sperm in the vaginal smear. If implantation did not occur, as indicated by the absence of erythrocytes in the smear on the fourteenth day of pregnancy, the rat was bred at the following estrus. Failure of implantation after positive mating

[3] Mead Johnson.

[4] Ephynal, of Hoffman-La Roche, Inc., through the courtesy of Dr. F. Gudernatsch.

[5] Merck and Co., through the courtesy of Dr. J. M. Carlisle.

occurred in forty (10%) out of 404 gestations under these conditions.

If the first gestation resulted in the birth of living young, the female was given diet II and permitted to suckle them. Record was made of the number of young which survived the first 10 days of lactation, and from the seventeenth to twenty-fifth day the rats were observed daily for symptoms of muscle dystrophy. Survivors were killed on the twenty-fifth day. Autopsies were performed upon every rat in the litter and representative muscle specimens preserved for histological examination. The diagnosis of muscle lesions was based upon microscopic evidence.

After the period of lactation the females were given diet I and bred again in 2 weeks. The second gestation was studied in the same way as the first. If litters were born, the rat was bred for the third time. All rats were bred until the occurrence of a resorption gestation, evidence for which was based upon the following findings: sperm in the vaginal tract, followed on the fourteenth day of gestation by the "implantation sign"; from the nineteenth to twenty-third day, a loss in body weight of not more than 5 gm. daily. Since the presence of resorption sites in the uterus was not confirmed directly, the possibility exists that some of the so-called resorptions may have been pseudo-pregnancies. However, in the latter case (Evans, '28), the "placental sign" usually occurs on the tenth or eleventh day, 2 or 3 days before this sign is found in true pregnancies.

In tests for the prevention of muscular dystrophy, female rats that had been used in the anti-sterility test, or young rats reared on diet I, were kept on diet I and bred. During the first 5 days of gestation 1 gm. of fresh wheat germ oil was given by mouth. All of the young born were kept for lactation, during which period the diet of the mother was changed to diet II. On the fifteenth to seventeenth day of lactation some of the young were given orally or parenterally single doses of α-tocopherol. The remaining young were kept as controls and were not given any supplementary feeding. During the rest

of the period of lactation, they were observed as described above. Litters in which the untreated controls did not develop muscle lesions were discarded.

RESULTS

Cure of sterility in the adult female rat

Fertility. As proposed by Bacharach and Allchorne ('38), this was expressed as the per cent of gestations which resulted in the birth of young, regardless of litter size or number of living young; furthermore, a loss in body weight of 15 gm. or more during the twenty-second day was assumed to indicate the casting of a litter.

TABLE 1

Prevention by α-tocopherol of resorption during gestation in rats on an E-low diet.

	α-TOCOPHEROL: SINGLE DOSE (IN MILLIGRAMS) AT THE BEGINNING OF THE FIRST GESTATION					
	0.5	1.0	2.5	10.0	20.0	50.0
Natural						
No. of gestations	3	14	8	4	3	—
No. of litters	0	11	8	3	3	—
Per cent fertility	0	79	100	75	100	—
Synthetic						
No. of gestations	3	14	5	4	3	2
No. of litters	0	8	5	4	3	2
Per cent fertility	0	57	100	100	100	100
Synthetic acetate						
No. of gestations	3	32	17	4	8	4
No. of litters	0	16	14	4	8	4
Per cent fertility	0	50	82	100	100	100

The fertility of females that subsisted on diet I and were fed single doses of α-tocopherol at the beginning of the first gestation is shown in table 1. It will be seen that the three preparations gave essentially the same results: 0.5 mg. of α-tocopherol was insufficient to permit the birth of a litter; 1.0 mg. induced litters in thirty-five (58%) of sixty rats and 2.5 mg. in twenty-seven (90%) of thirty rats; at 10, 20 and 50 mg., the incidence was nearly 100%. The biological variation was very great; for instance, among fifteen groups of

rats receiving 1.0 mg. of α-tocopherol there were some that had no young, while in others, all of the rats cast litters.

The minimum amount of α-tocopherol which permitted the birth of young to at least 85% of rats under the given experimental conditions was approximately 2.5 mg. This value lies within the limits, 1.0 to 3.0 mg., given for the minimum dose by Evans, Emerson and Emerson ('36), Karrer and Keller ('38), and Bacharach ('38).

TABLE 2

Influence of α-tocopherol in rats on an E-low diet upon litter size and the number of young born alive, surviving the early lactation period, and developing muscular dystrophy.

	MILLIGRAMS OF α-TOCOPHEROL: SINGLE DOSE AT BEGINNING OF GESTATION				
	1.0	2.5	10.0	20.0	50.0
No. of litters	28	22	11	13	5
No. of young	194	190	97	111	43
Av. young per litter	6.9	8.6	8.8	8.5	8.6
No. living young	131	163	90	109	43
Per cent	67	86	93	99	100
No. surviving 10 days	61	95	22	78	29
Per cent	46	58	24	71	67
No. kept for muscular dystrophy study (not given α-tocopherol)	16	28	18	70	29
No. with muscular dystrophy	16	25	15	65	22
Per cent	100	89	83	93	76

Litter size. The litters varied in size from one to fourteen rats. It will be seen in table 2 that in rats receiving 1.0 mg. of α-tocopherol the average number of young per litter was 6.9. At 2.5 mg., the litter size averaged 8.6. Larger amounts of α-tocopherol did not influence size, which results are in agreement with those reported by Evans and Burr ('27) for wheat germ oil.

Number of living young. In litters of rats receiving 1.0 mg. of α-tocopherol, 131 (67%) of 194 young were living at the time that the litters were found. As the amount of α-tocopherol was increased up to 10 mg., the number of living young increased to 90%. Twenty and 50 mg. were no more effective than 10 mg.

Although no systematic study has been made of the newborn rats, dystrophic lesions of the skeletal muscles were discovered in nine of twenty-three rats dying or killed on the first day. Oedema, with or without necrosis of muscle fibers, was noted in a large proportion of these newborn rats.

Number of second and third litters. The birth of second litters after one dose of vitamin E has been reported by Evans and Burr ('27) and others. The number of second and third litters born after a single dose of α-tocopherol at the beginning of the first gestation is shown in table 3. Since there was no apparent difference between them, the data obtained from the three separate preparations were combined in this and the

TABLE 3

Number of litters occurring in rats on an E-low diet after a single dose of α-tocopherol at the beginning of the first gestation.

NUMBER OF LITTERS.	MILLIGRAMS OF α-TOCOPHEROL					
	0.5	1.0	2.5	10.0	20.0	50.0
1	0	12	13	10	14	6
2		3	4	5	6	1
3		1	0	2	1	1
4		0		0	0	0

following tables. It will be seen that as the α-tocopherol was increased in amount from 1.0 to 10 mg., the proportion of second litters became greater, but at no level was it higher than 50%. Third litters occurred but rarely, even at 20 and 50 mg., and there were no fourth litters.

From the evidence it appears that the ability of female rats to produce more than one litter after a single dose of α-tocopherol is limited and that the saturation point is reached at approximately 5 to 10 mg. These results are not entirely in accord with those of Evans and Burr ('27) who state that "the administration of the minimal effective dose of E never leads to the survival of fertility beyond the trial gestation for which it was employed", or of Olcott and Mattill ('34) who report that a single large dose of vitamin E allows fertility for two gestations but not for three.

Success of lactation

Survival of young. The number of young which survived the first 10 days of lactation is given in table 2. It will be seen that about half of the young of litters of females receiving 1.0 to 10 mg. of α-tocopherol at the beginning of gestation survived. At 20 to 50 mg. about 70% survived. These results are in agreement with those of Sure ('26) who reported upon the existence of a factor in the unsaponifiable matter from wheat germ oil, necessary for successful lactation.

The cause of death of the young rats from the second to the tenth day was not determined; only in a few instances, were there any muscle lesions.

Incidence of muscular dystrophy. In table 2 is shown the incidence of muscular dystrophy in the young of females receiving single doses of α-tocopherol at the beginning of gestation. At 1.0 mg., all of the young (sixteen) developed the disorder. With larger doses there was some protection, but even at 50 mg. the incidence of muscle disease was 76%. These results indicate that the capacity of female rats to store vitamin E given at the beginning of gestation, to transfer it through the placenta, and to excrete it in the milk, is so limited that their young are not protected against muscle dystrophy. The disease is prevented in all of the young if 10 mg. of α-tocopherol are given to the female at the time of parturition (Evans and Emerson, '40).

Prevention of muscular dystrophy in the young rat

Preparation of test animal. The reproductive performance of female rats on diet I with 1 gm. of fresh wheat germ oil during the first 5 days of each gestation is given in table 4. The data are arranged according to the number of the gestations. Two hundred and thirty-seven gestations resulted in 214 litters, a fertility of 90%. The fertility of the rats during the first three gestations averaged more than 90%. In subsequent gestations, when the rat was 10 months of age or older, the fertility decreased, as reported by Olcott and Mattill ('34) and Emerson and Evans ('39).

The size of the litter tended to decrease in older animals, but the proportions of living young remained constant (averaging 90%). The numbers of living young were distributed among litters as follows: of 214 litters, there were 156 (73%) in which all of the young were living; 45 (22%) in which some of the young were living; and 12 (5%) in which none were living.

TABLE 4

Reproduction of female rats on diet I with 1 gm. fresh wheat germ oil at the beginning of gestation.

NUMBER OF GESTATION	1	2	3	4	5	6	TOTALS
No. of gestations	12	70	87	50	14	4	237
No. of litters	12	66	80	41	12	3	214
Per cent fertility	100	94	92	82	86	75	90
No. of young	86	537	565	244	68	10	1510
Av. young per litter	7.2	8.1	7.1	6.0	5.7	3.3	7.0
No. living young	69	497	528	207	59	10	1370
Per cent	80	92	93	85	87	100	91
No. surviving 10 days	51	401	432	177	52	8	1121
Per cent	74	81	82	85	88	80	82
No. kept for muscular dystrophy study (not given α-tocopherol)	22	190	206	86	22	4	530
No. with disease	22	162	184	83	19	2	472
Per cent	100	85	88	96	86	50	89

The number of young surviving 10 days of lactation also remained constant (averaging 80%). They were distributed among litters as follows: of 202 litters of living young, there were 93 (46%) in which all of the young survived; 75 (37%) in which part of the young survived; and 34 (17%) in which none of the young survived. The number of young surviving 10 days of lactation was greater than had been observed upon 50 mg. of α-tocopherol (see table 2).

However, in forty-four females on diet I that were fed 20 mg. of α-tocopherol at the beginning of gestation (not the first gestation), forty-three (98%) had litters; there were 334 young (average 8.0 per litter), of which 311 (93%) were

living; 259 (83%) survived. These results compare favorably with those of the rats receiving wheat germ oil.

The incidence of muscular dystrophy at the end of lactation among the young rats is shown in table 4. Four hundred and seventy-two (89%) of 530 young developed the disorder. The rats with muscle lesions were distributed in litters as follows: of 165 litters in which four or more rats survived 10 days of lactation, there were 129 (78%) in which all of the rats presented degenerated muscles; 30 (18%) in which part of the young were affected and 6 (4%) in which none of the rats developed muscular dystrophy.

The occurrence of muscular dystrophy was determined by microscopic examination. In 863 rats with well-defined pathological lesions, 540 (63%) presented typical symptoms, and 805 (94%) presented gross lesions. Thus about a third of the animals had muscle lesions without symptoms. Calcification was observed in 72 (8%) of muscles. In 86 rats with slight microscopic lesions, 20 (23%) presented symptoms and only 27 (31%) presented gross pathological changes. Although the disease usually appeared on the nineteenth to twenty-third day of life, in one litter lesions were found in rats only 13 days of age.

Prevention of muscular dystrophy in the young rat. Muscular dystrophy is prevented in young rats of females on vitamin E-low diets by the feeding daily after the tenth day of lactation of small amounts of α-tocopherol (Goettsch and Ritzmann, '39; Evans and Emerson, '40). In preliminary experiments single doses of approximately 0.5 mg. proved to be effective.

In order to determine at what time of lactation it was best to give the vitamin, single doses of 0.5 to 1.0 mg. of α-tocopherol were fed to the young on the tenth to nineteenth day. The results are given in table 5. The greatest number of young were protected when the vitamin was fed on the fifteenth to seventeenth day. When fed at this time 0.5 mg. was as effective as 1.0 mg.

Varying amounts of the three preparations of α-tocopherol were fed on the fifteenth to sixteenth day. It will be seen in table 6 that there were apparently no essential differences between the potencies of the natural and synthetic products. The minimum amount of α-tocopherol which prevented muscular dystrophy in at least 85% of young under the given experimental conditions was approximately 0.5 mg.

TABLE 5

Prevention of muscular dystrophy in the young rat by single doses of α-tocopherol on the tenth to the nineteenth day of lactation

DAY OF LACTATION	MILLIGRAMS α-TOCOPHEROL					
	1.0			0.5		
	No. of rats	No. with muscular dystrophy	%	No. of rats	No. with muscular dystrophy	%
10–11	42	7	17	36	11	30
12–14	12	1	8	21	3	14
15–17	49	1	2	54	1	2
18–19	19	0	0	11	2	18

TABLE 6

Prevention of muscular dystrophy in the young rat by the feeding of single doses of α-tocopherol on the fifteenth to sixteenth day of lactation.

α-TOCOPHEROL		NUMBER OF RATS	NUMBER WITH MUSCULAR DYSTROPHY	PER CENT
	mg. 0	278	255	92
Natural	0.25	9	3	33
Synthetic		8	3	38
Synthetic acetate		9	6	67
Natural	0.50	9	2	22
Synthetic		8	1	12
Synthetic acetate		85	8	9
Natural	1.00	18	0	0
Synthetic acetate		70	1	1.4
Synthetic acetate	2.00	6	0	0
Synthetic acetate	3.00	11	0	0

An assay method based upon the cure of muscle dystrophy in the young rat is not feasible because of the spontaneous recovery of about 65% of the affected young (Evans and Burr, '28; Goettsch and Ritzmann, '39).

Relative ineffectiveness of parenteral administration. Litter mates reared on diet I were divided into three groups. At the beginning of gestation one group was fed 1.0 mg. of α-tocopherol (natural or the synthetic acetate), the second group was fed 2.5 mg., and the third group was given 2.5 mg. by subcutaneous injection. The results are shown in table 7. It will be seen that the rats receiving 2.5 mg. of α-tocopherol subcutaneously responded in approximately the same way with respect to fertility, litter size, number of living young, and lactation as the rats that were fed 1.0 mg.

TABLE 7

Ineffectiveness of parenteral administration of α-tocopherol in the prevention of sterility in the female rat.

	MILLIGRAMS OF α-TOCOPHEROL: SINGLE DOSE AT BEGINNING OF GESTATION		
	2.5	1.0	2.5
	Fed	Fed	Injected subcutaneously
No. of gestations	7	8	10
No. of litters	7	5	5
Per cent fertility	100	62	50
No. of young	67	36	39
Av. young per litter	9.6	7.2	7.8
No. living young	63	15	30
Per cent	94	42	77
No. surviving 10 days	42	12	10
Per cent	67	80	33
No. kept for muscular dystrophy study (not given α-tocopherol)	19	12	10
No. with disease	16	12	10
Per cent	84	100	100

Young rats of females on diet I were given α-tocopherol (natural or synthetic acetate) orally or parenterally on the fifteenth to seventeenth day of lactation. By separating the young for several hours it was possible to prevent the mother rats from obtaining α-tocopherol from the sites of injection. It will be seen in table 8 that although the young which were fed 0.5 or 1.0 mg. of α-tocopherol were protected, their litter mates which received the same amounts by injection developed

muscle lesions. The parenteral administration of larger amounts of α-tocopherol afforded some protection but the injection of 10 mg. subcutaneously or intraperitoneally was not so effective as the feeding of 0.5 mg.

TABLE 8

Failure to prevent muscular dystrophy in the young rat by the injection of single doses of α-tocopherol on the fifteenth to seventeenth day of lactation.

	α-TOCOPHEROL	NUMBER OF RATS	NUMBER WITH MUSCULAR DYSTROPHY	PER CENT
	mg.			
Untreated controls	0	65	61	94
Subcutaneous injection	0.5	6	6	100
	1.0	17	15	88
	2.5	12	9	75
	5.0	9	6	67
	10.0	8	5	62
Intraperitoneal injection	0.5	2	2	100
	1.0	13	12	92
	2.5	6	6	100
	5.0	10	6	60
	10.0	8	3	38
Oral	0.5	17	1	6
	1.0	27	0	0

SUMMARY

1. Under the experimental conditions described, 2.5 mg. of α-tocopherol fed at the beginning of gestation permits the birth of young to at least 85% of female rats on vitamin E-low diets. The litters are of normal size (average 8.6 young), there are at least 85% of living young, 60% of which survive the first 10 days of lactation. Of the surviving rats, 90% develop muscular dystrophy at the end of lactation. Second litters are born to about one-fourth of the females.

2. Under the experimental conditions described, 0.5 mg. of α-tocopherol fed on the fifteenth to the seventeenth day of lactation prevents muscular dystrophy in at least 85% of the young of female rats on vitamin E-low diets.

3. There are no essential differences in the anti-sterility and anti-muscular dystrophy preventing potencies between natural or synthetic α-tocopherol or the synthetic acetate.

4. The α-tocopherol must be fed as it is not so active when administered parenterally.

5. Muscular dystrophy may occur in the newborn rat.

LITERATURE CITED

BACHARACH, A. L. 1938 Investigations into the method of estimating vitamin E. III. The relation between dosage and response to vitamin E. Biochem. J., vol. 32, p. 2017.

BACHARACH, A. L., AND E. ALLCHORNE 1938 Investigations into the method of estimating vitamin E. II. Further observations on vitamin E deficiency and implantation. Biochem. J., vol. 32, p. 1298.

EMERSON, G. A., AND H. M. EVANS 1939 Restoration of fertility in successively older E-low female rats. J. Nutrition, vol. 18, p. 501.

EVANS, H. M. 1928 Spontaneous deciduomata in pseudopregnancy with low vitamin E. Amer. J. Physiol., vol. 85, p. 149.

EVANS, H. M., AND K. S. BISHOP 1923 On the relation between fertility and nutrition. IV. The production of sterility with nutritional regimes adequate for growth. J. Metabolic Res., vol. 3, p. 233.

EVANS, H. M., AND G. O. BURR 1927 The antisterility vitamine fat soluble E. Memoirs of the University of California, Berkeley, California, vol. 8.

———— 1928 Development of paralysis in the suckling young of mothers deprived of vitamin E. J. Biol. Chem., vol. 76, p. 273.

EVANS, H. M., AND G. A. EMERSON 1940 Prevention of nutritional muscular dystrophy in suckling E-low rats with α-tocopherol and related substances. Proc. Soc. Exper. Biol. and Med., vol. 44, p. 636.

EVANS, H. M., O. H. EMERSON AND G. A. EMERSON 1936 The isolation from wheat germ oil of an alcohol, α-tocopherol, having the properties of vitamin E. J. Biol. Chem., vol. 113, p. 319.

GOETTSCH, M., AND J. RITZMANN 1939 The preventive effect of wheat germ oils and of α-tocopherol in nutritional muscular dystrophy of young rats. J. Nutrition, vol. 17, p. 371.

HAWK, P. B., AND B. L. OSER 1931 A modification of the Osborne-Mendel salt mixture. Science, vol. 74, p. 369.

KARRER, P., AND H. KELLER 1938 Quantitative Bestimmung der Tocopherole in verschiedenen Ausgangsmaterialen. Helv. chim. Acta, vol. 21, p. 1161.

MASON, K. E. 1940 Minimal requirements of male and female rats for vitamin E. Am. J. Physiol., vol. 131, p. 268.

MASON, K. E., AND W. L. BRYAN 1938 Standardization of the rat for bio-assay of vitamin E. Biochem. J., vol. 32, p. 1785.

MATTILL, H. A., AND N. C. STONE 1923 II. The nutritive properties of milk with special reference to reproduction in the albino rat. J. Biol. Chem., vol. 55, p. 443.

OLCOTT, H. S. 1938 The paralysis in the young of vitamin E deficient female rats. J. Nutrition, vol. 15, p. 221.

OLCOTT, H. S., AND H. A. MATTILL 1934 Vitamin E. I. Some chemical and physiological properties. J. Biol. Chem., vol. 104, p. 423.

PALMER, L. S. 1937 Biological assay of vitamin E; application to wheat germ and wheat germ oil. Indust. Eng. Chem. Anal. Ed., vol. 9, p. 427.

SURE, B. 1923–1924 II. The existence of a specific vitamin for reproduction. J. Biol. Chem., vol. 58, p. 693.

———— 1926 Dietary requirements for reproduction. VII. The existence of a lactation-promoting factor in the unsaponifiable matter from wheat germ oil. J. Biol. Chem., vol. 69, p. 53.

THE UTILIZATION OF THE CALCIUM OF CAULIFLOWER AND BROCCOLI

MARGARET L. FINCKE
School of Home Economics, Oregon State College, Corvallis

(Received for publication July 24, 1941)

The availability of the calcium of several vegetables has been studied in various laboratories. Fincke and Sherman ('35) reviewed the literature on the subject up to 1935. They reported that the calcium of kale was practically as well utilized as that of milk, whereas the calcium of spinach was poorly utilized by young growing rats, if, indeed, it was utilized at all. The poor utilization of the calcium of spinach was due, in part at least, to the high oxalate content. Fairbanks and Mitchell ('38) confirmed these findings for spinach.

Kung, Yeh and Adolph ('38) studied several Chinese vegetables and found that with the exception of spinach and soybean sprouts, all caused high calcium retention values, which were comparable with those obtained with skim milk powder. Kao, Conner and Sherman ('38) reported that the availability of the calcium of Chinese cabbage was 90% as high as that of milk. Speirs ('39) found the calcium of turnip greens to be as available as that of milk and that of tendergreen, collards and kale somewhat less. The calcium of New Zealand spinach not only was poorly utilized by rats, if at all, but the utilization of the calcium of milk in the diet was diminished by the presence of the New Zealand spinach. The dried New Zealand spinach contained 4.8% oxalate. More recently this author ('40) has suggested that some factor other than oxalic acid may be concerned with this poor utilization.

The calcium of carrots, lettuce, and string beans was reported by Shields, Fairbanks, Berryman and Mitchell ('40) to be somewhat less available than that of milk. With milk solids, 77 to 83% of the calcium was retained, while 71% of the calcium of carrots, 61% of that of lettuce, and 60% of the calcium of string beans was utilized, with no difference being observed between fresh and cooked vegetables so far as calcium utilization was concerned.

This investigation was undertaken to study the utilization of calcium of two vegetables which are flowers, i.e., cauliflower and sprouting broccoli.

EXPERIMENTAL

The method here used is essentially that carried out by Fincke and Sherman ('35). Healthy young rats were weaned at 28 days and placed on the experimental diets, the compositions of which are given in table 1.

TABLE 1

Percentage composition of diets, and calcium content.

	DIET 108	DIET 109	DIET 110
Whole wheat	44.5	44.5	44.5
Skim milk powder	20.0	10.0	10.0
Butterfat	10.0	10.0	10.0
Cornstarch	24.5	20.5	—
Dried broccoli	—	14.0	—
Dried cauliflower	—	—	34.5
Sodium chloride	1.0	1.0	1.0
Calcium content in %	0.277	0.267	0.267

Litter mates of the same sex were used in comparing the three diets, one animal from each litter being placed on each diet and one killed at 28 days of age for analysis. The initial weights of the animals were kept as nearly alike as possible. Each group consisted of five males and five females. The diets were fed ad libitum, records being kept of the amounts eaten until the animals were 60 days of age, when they were killed by chloroform. In previous unpublished work, no differences

were observed between the results obtained with ad libitum feeding and paired feeding. The bodies of the animals minus the digestive tract were ashed and analyzed for calcium by a modification of the McCrudden method.

Those parts of cauliflower and broccoli used for human consumption were prepared in amounts sufficient to last through the whole experiment as follows: washed well in tap and then distilled water, dried in a recirculating air drier operated at temperatures not exceeding 60°C., ground and thoroughly mixed. Each vegetable was carefully sampled and analyzed for calcium.

RESULTS

The comparative growth of the animals is given in table 2. Growth of the rats which were fed the diet containing broccoli amounted to 0.255 gm. per gram of food for the males, and 0.226 gm. per gram of food for the females. These gains were somewhat less than those of the rats fed the control diet (0.285 gm. and 0.248 gm. per gram of food for the males and

TABLE 2

Growth and calcium content of animals receiving diets containing milk, or milk plus broccoli or cauliflower as chief sources of calcium. All values are averages.

DIET	SEX	BODY WEIGHT		FOOD EATEN	GROWTH PER GRAM OF FOOD EATEN	CALCIUM			
		Initial	Final			Body content		Ingested	Utilization factor
						Amount	Per cent		
		gm.	*gm.*	*gm.*	*gm.*	*gm.*		*gm.*	
108	♂ (5)[1]	49.4	150.4	355	0.285	1.275	0.848	0.982	0.87
	♀ (5)	49.6	131.6	331	0.248	1.224	0.932	0.917	0.87
									0.87±0.017[2]
109	♂ (5)	49.8	137.4	344	0.255	1.151	0.839	0.918	0.79
	♀ (5)	49.8	123.8	328	0.226	1.111	0.898	0.877	0.79
									0.79±0.018[2]
110	♂ (5)	49.8	111.6	288	0.212	0.989	0.892	0.769	0.73
	♀ (5)	50.4	99.6	272	0.180	0.916	0.920	0.728	0.66
									0.69±0.020[2]

[1] Number of animals indicated in parentheses.

[2] Average and standard error of the mean.

females respectively), although food consumption was practically the same. The difference is not statistically significant, however. Food consumption and growth were significantly lower on the diet containing cauliflower than on the control diet, the growth per gram of food being 0.212 gm. for the males and 0.180 gm. for the females, or 26% less on the cauliflower diet. Probably some of this difference was due to the presence of fiber, as less efficient growth on diets containing fiber was observed by Fincke ('35). In those studies the gain per gram of food was depressed by 22% by the simple addition of 1.2% washed fiber to the diet consisting of two-thirds whole wheat, one-third whole milk powder.

The utilization of calcium of the different diets is shown in table 2. The calcium utilization factor here given is obtained by dividing the amount of calcium stored during the 32 days on the diet by the amount of calcium ingested. The storage of calcium represents the amount of calcium in the body of the rat at death minus the amount in the body at 28 days of age, calculated from the average calcium content of the 28-day old rats of the same sex which were killed for analysis at that age. The males at this age contained on the average 0.861% and the females 0.865% calcium.

Little difference is evident in the average percentages of calcium in the bodies of the rats on the three different diets. However, the animals receiving the broccoli or cauliflower diets were less efficient in their use of calcium, the differences being statistically significant. The milk calcium apparently was 87% available, that of the broccoli plus milk was 79% available, and that of the cauliflower plus milk only 69% available. In other words, the calcium utilization factor of the broccoli diet was 8.9% lower than that of the milk diet, and that of the cauliflower diet was 19.1% less than that of the milk diet. On comparing the percentage utilization of calcium of milk here obtained with the figures reported by Fincke and Sherman (0.81 for males and 0.75 for females), by Fairbanks and Mitchell (0.90), by Shields, Fairbanks, Berryman and Mitchell (0.668 to 0.83), and by Speirs (0.80 and 0.78), it will

be seen that the figures here found are in the same range as those reported by other investigators.

The dried broccoli contained 0.38% oxalic acid and the dried cauliflower 0.24%, determined by a modification of Bau's method ('19). If these amounts of oxalate rendered equivalent amounts of calcium unavailable, the calcium utilization factors of the diets containing broccoli and cauliflower calculated on the basis of available rather than total calcium would average 0.80 for each diet, which is still somewhat below the value here obtained for the milk diet.

The crude fiber content of dried broccoli and cauliflower was determined by the A.O.A.C. method ('40) and was found to be 9.7% for the broccoli and 9.6% for the cauliflower. The cauliflower diet, however, contained much more dried vegetable than the broccoli diet, so that the fiber content of that diet was higher. Adolph, Wang and Smith ('38) reported that loss of calcium in the feces when large quantities of fiber were fed amounted to less than 10%. Former work by the author, including some unpublished data, showed a barely significant lowering of the availability of the calcium due to the presence of added fiber, the lowering amounting to about 8%. If the calcium utilization factor here found for the milk diet, 0.87, were lowered by 8%, it would result in a factor of 0.80. The combination of the fiber and the oxalate contents of the broccoli and cauliflower diets could, therefore, account for the total lowering of the availability of the calcium of these two diets, and may actually have been the causes of poorer utilization of the calcium of broccoli and cauliflower.

SUMMARY AND CONCLUSIONS

Young rats in strict litter mate comparison were fed diets in which practically all of the calcium was derived from dried milk, or in which half of the milk was replaced by dried broccoli or cauliflower to provide approximately the same amounts of calcium. At 60 days of age they were killed and their bodies analyzed for calcium.

The calcium utilization factor for the milk diet amounted to 0.87 ± 0.017; of the diet containing broccoli to 0.79 ± 0.018, and of the diet containing cauliflower to 0.69 ± 0.020. The possible causes of this lower availability of the calcium of cauliflower and broccoli are discussed.

LITERATURE CITED

ADOLPH, W. H., C. H. WANG AND A. H. SMITH 1938 The effect of roughage on the calcium balance in rats. J. Nutrition, vol. 16, pp. 291–297.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1940 Methods of Analysis, 5th edition.

BAU, A. 1919 Determination of oxalic acid. Wochschr. Brau., vol. 36, p. 293; vol. 37, p. 201.

FAIRBANKS, B. W., AND H. H. MITCHELL 1938 Availability of calcium in spinach, in skim milk powder and in calcium oxalate. J. Nutrition, vol. 16, pp. 79–89.

FINCKE, M. L. 1935 The availability of calcium from some typical foods. Dissertation, Columbia University, New York.

FINCKE, M. L., AND H. C. SHERMAN 1935 The availability of calcium from some typical foods. J. Biol. Chem., vol. 110, pp. 421–428.

KAO, H. C., R. T. CONNER AND H. C. SHERMAN 1938 The availability of calcium from Chinese cabbage (Brassica pekinensis, Rupr.). J. Biol. Chem., vol. 123, pp. 221–228.

KUNG, L. C., H. L. YEH AND W. H. ADOLPH 1938 The availability of calcium in vegetable food materials. Chinese J. Physiol., vol. 13, pp. 307–316. Nutr. Abs. Rev., vol. 8, p. 697 (1939).

SHIELDS, J. B., B. W. FAIRBANKS, G. H. BERRYMAN AND H. H. MITCHELL 1940 The utilization of calcium in carrots, lettuce and string beans in comparison with the calcium in milk. J. Nutrition, vol. 20, pp. 263–278.

SPEIRS, MARY 1939 The utilization of the calcium in various greens. J. Nutrition, vol. 17, pp. 557–564.

———— 1940 The availability of calcium in various foods. In Notes on Graduate Studies and Research in Home Economics and Home Economics Education, 1939–1940, U. S. Department of Agriculture.

THE EFFECT OF INCUBATION ON THE VITAMIN CONTENT OF EGGS

ESMOND E. SNELL AND ERNESTINE QUARLES
Department of Chemistry, University of Texas, Austin

(Received for publication July 18, 1941)

Almost no work has been reported on the changes which occur in the vitamin content of hen eggs during incubation and hatching. Suomalainen ('39 a) found that the vitamin A content of incubated eggs decreased by 45% during the second and third weeks of incubation, while the carotene remained unchanged. The same author ('39 b) followed the changes in ascorbic acid content of eggs during incubation. Ascorbic acid was absent in the unhatched egg, but increased greatly during the first 2 weeks of incubation. Since the chick requires vitamin A in its diet, but is able to synthesize its own supplies of vitamin C, these are the changes which might have been expected. At the same time, however, such studies afford a means for determining which of the other vitamins can be synthesized by the developing chick embryo and presumably, therefore, are not required in the diet of the chick.

In the present study the total concentrations of riboflavin, pantothenic acid, nicotinic acid, biotin and inositol in the egg have been followed from the beginning of incubation to the time of hatching. Evidence is abundant and conclusive that riboflavin and pantothenic acid are required in the diet of chicks, and for development of the chick embryo (Lepkovsky et al., '38; Bauernfeind and Norris, '39; Jukes, '37), while biotin is presumably also required (Ringrose et al., '31; Hegsted et al., '40). No effect of nicotinic acid on chick growth has been observed (Mickelsen et al., '38). Inositol, recently identi-

fied as a dietary essential for the mouse (Woolley, '40), has not been studied in this regard. Pantothenic acid and riboflavin have therefore been used to indicate the changes which may occur in the concentration of an essential dietary constituent during embryonic development, and changes in the other constituents studied have been compared with these.

EXPERIMENTAL

The eggs used were from a private flock of White Leghorn breeders maintained near Austin, and showed uniformly high hatchability. The weighed eggs were marked and incubated at 38.9–39.5°C. in electrical units. At 3- to 4-day intervals throughout the incubation period eight eggs which showed normal embryonic development were taken. These were divided into four sets of two eggs each. The contents of the two eggs of each set were removed from the shells, combined, an equal weight of water added, and the sample homogenized in the Waring Blendor. All samples were then allowed to autolyze under benzene at 37°C. for 24 hours. Both pantothenic acid (Rohrmann et al., '34) and biotin (Snell et al., '40) occur in tissues in a bound form liberated by autolysis. While this procedure was superfluous at the beginning of incubation it becomes increasingly necessary as the embryo develops, and was carried out in all cases to insure reproducibility of results. At the end of this time all samples were diluted to contain the equivalent of 200 mg. of original sample (including shell) per cubic centimeter. Since eggs lose considerable weight during incubation (compare Pringle and Barott, '37) all weights were expressed as the original egg weight before incubation. Aliquots were autoclaved at 15 pounds steam pressure for 20 minutes, then rediluted with water to the original volume. Glacial acetic acid was added dropwise with shaking until the coagulated material became flocculent and easily filtered. A portion of the filtrate was saved for analysis.

At the end of 20 days incubation some chicks were beginning to hatch. Unhatched chicks were taken for the 20-day

group. The hatched chicks taken at 23 days were from eggs of known weight and had not yet received food or water. Samples were prepared from the whole chicks as described above, except that the chick was decapitated and ground before being placed in the Blendor. The average weight of the chicks of this group was 34.3 gm.; the average original weight of the eggs (before incubation) from which they were hatched was 57.5 gm.

Riboflavin was determined by the microbiological method (Snell and Strong, '39). Pantothenic acid was determined by the method of Snell, Strong and Peterson ('37) as modified by Pennington et al. ('40). Several samples were also assayed by the modification of the former method presented by Strong, Feeney and Earle ('40) with identical results. Nicotinic acid was determined microbiologically with *Lactobacillus arabinosus* (Snell and Wright, '41). Biotin was determined with this same organism, as recommended in the latter reference. A sample of biotin methyl ester (kindly furnished by Doctor Du Vigneaud) was hydrolyzed with alkali and used as the biotin standard. Inositol was determined by a yeast growth method (Williams et al., '41).

Results are given in table 1. Pantothenic acid remained constant throughout the incubation period. Riboflavin likewise remained constant. The small apparent increase in riboflavin during the first 4 to 7 days of incubation represented an extraction difficulty during the first few days, since direct assays made by adding the mixed original sample (without heating or filtering) to the assay tube gave figures in good agreement with those obtained from 7 days on. Thus two vitamins of known essential nature for the chick remain constant during incubation, neither increasing nor decreasing in total concentration. In marked and unmistakable contrast, nicotinic acid rises steadily from a low initial value to a level more than twenty times higher in the hatched chick. This demonstrates conclusively that the chick embryo synthesizes its own nicotinic acid, and that bacterial synthesis is not involved. Presumably this synthetic power is retained by the

TABLE 1

Effect of incubation on the vitamin content of eggs.

INCUBATION TIME	EGG WEIGHT PER PAIR	PANTOTHENIC ACID	RIBOFLAVIN	NICOTINIC ACID	INOSITOL	BIOTIN
days	*gm.*	*μg. per gm.*	*μg. per gm.*	*μg. per gm.*	*μg. per gm.*	*mγ per gm.*
0	120	12	2.0	0.77	74	59
	117	11	1.9	0.73	64	53
	117	10	2.3	0.90	73	70
	116	12	1.9	1.1	82	90
Average	118	11	2.0	0.85	73	68
4	113	12	3.2	1.1	57	50
	113	12	2.4	0.90	66	51
	117	13	2.5	0.90	69	50
	117	10	2.3	1.1	67	57
Average	115	12	2.6	1.0	65	52
7	117	14	3.0	2.7	66	92
	116	14	3.6	2.8	70	67
	113	13	3.4	3.0	78	69
	115	12	3.3	2.8	85	82
Average	115	13	3.3	2.8	75	78
10	119	14	3.5	3.1	88	99
	115	12	3.1	2.6	95	53
	117	13	3.0	2.6	89	86
	115	10	2.9	3.4	88	75
Average	116	12	3.1	2.9	90	78
13	119	14	2.9	6.9	104	62
	116	12	3.0	7.4	119	53
	115	12	2.8	8.0	121	54
	117	13	3.4	7.1	128	44
Average	117	13	3.0	7.3	118	53
17	118	11	3.0	13	200	46
	115	11	3.2	15	224	57
	116	13	3.4	17	208	76
	118	10	2.7	11	167	26
Average	117	11	3.1	14	200	51
20	116	11	3.2	17	269	64
	118	14	3.0	16	264	36
	119	16	3.8	15	266	68
	119	12	2.9	15	254	60
Average	118	13	3.2	16	263	57
23	111	10	3.3	16	445	26
	116	13	3.3	20	468	23
	116	11	2.9	19	490	29
	116	14	3.5	20	420	22
Average	115	12	3.3	19	456	25

chick. All efforts to raise the apparent nicotinic acid content of unincubated egg, or to alter the final value by acid or alkaline hydrolysis of the samples met with failure. Kringstad and Thoresen ('40) found by chemical assay only traces of nicotinic acid in hen eggs, although the flesh of hens was twice as rich in nicotinic acid as was beef. Dann and Handler ('41) have recently reported marked increases in the nicotinic acid content of chick breast muscle during the first 3 months after hatching. Shourie and Swaminathan ('40) have shown by balance experiments that rats do not require nicotinic acid in the diet. The possibility that it was furnished by bacterial action in the intestinal tract, and not synthesized by the tissues could not be excluded by this method.

In a manner similar to nicotinic acid, inositol increased regularly in concentration from about the seventh day of incubation on, to a final level about six times that present in the original egg. These results would indicate that the chick embryo is likewise capable of synthesizing inositol.

The biotin content of eggs was more variable than was any other constituent which was determined. The average biotin content, however, showed no tendency to increase during incubation, and decreased after hatching. The decrease is only apparent, however, since acid hydrolysis of the samples after autolysis liberated additional biotin to an average value of 42 mγ per gram. Acid or alkaline hydrolysis of the control egg samples did not increase their biotin content. From these results it is evident that the chick embryo does not synthesize biotin. This result might indicate merely that sufficient biotin was already present in the egg for development. The observation that the biotin level in chick tissues is lowered when biotin of the diet is rendered unavailable by egg white (Eakin, McKinley and Williams, '40) indicates that it is a true vitamin for the chick, and could not be synthesized under any conditions. Recent direct evidence (Hegsted et al., '40) also supports this conclusion.

SUMMARY AND CONCLUSIONS

The total amount of pantothenic acid and of riboflavin present in hen eggs does not change during incubation. The amount of biotin also remains constant or decreases somewhat. Nicotinic acid is synthesized by the chick embryo, so that the final amount present in the newly-hatched chick is over twenty times that originally present in the unincubated egg. Inositol likewise appears to be synthesized by the chick embryo. Presumably these synthetic powers of the embryo are retained by the chick. If this is so, it would indicate that nicotinic acid and inositol, unlike riboflavin, pantothenic acid and biotin, are not required in the diet of the chick. Whether such synthetic powers are sufficient to supply the needs of the chick for optimal or maximal growth is, of course, not determined by the present data.

Generous support is acknowledged from funds granted to Prof. Roger J. Williams by the Rockefeller Foundation. The authors wish also to thank Mr. Raymond McMahan, Dr. L. D. Wright, and Dr. Alfred Taylor for assistance in certain parts of the work.

LITERATURE CITED

BAUERNFEIND, J. C., AND L. C. NORRIS 1939 The role of the antidermatosis vitamin and a new water soluble growth factor in the nutrition of the mature fowl. J. Nutrition, vol. 18, p. 579.

DANN, W. J., AND P. HANDLER 1941 The quantitative estimation of nicotinic acid in animal tissues. J. Biol. Chem., vol. 140, p. 201.

EAKIN, R. E., W. A. MCKINLEY AND R. J. WILLIAMS 1940 Egg-white injury in chicks and its relationship to a deficiency of vitamin H (biotin). Science, vol. 92, p. 224.

HEGSTED, D. M., J. J. OLESON, R. R. MILLS AND C. A. ELVEHJEM 1940 Studies on a dermatitis in chicks distinct from pantothenic acid deficiency. J. Nutrition, vol. 20, p. 599.

JUKES, T. H. 1937 Further observations on the assay, distribution, and properties of the filtrate factor. J. Biol. Chem., vol. 117, p. 11.

KRINGSTAD, H., AND F. THORESEN 1940 The occurrence of the antipellagra vitamin, nicotinic acid (-amide), in some foods. Nord. Med., vol. 8, p. 2248.

LEPKOVSKY, S., L. W. TAYLOR, T. H. JUKES AND H. J. ALMQUIST 1938 Effect of riboflavin and the filtrate factor on egg production and hatchability. Hilgardia, vol. 11, p. 559.

MICKELSEN, O., H. A. WAISMAN AND C. A. ELVEHJEM 1938 The inactivity of nicotinic acid in chick dermatitis. J. Biol. Chem., vol. 124, p. 313.

PENNINGTON, D. E., E. E. SNELL AND R. J. WILLIAMS 1940 An assay method for pantothenic acid. J. Biol. Chem., vol. 135, p. 213

PRINGLE, E. M., AND H. G. BAROTT 1937 Loss of weight of hen eggs during incubation under different conditions of humidity and temperature. Poultry Sci., vol. 16, p. 49.

RINGROSE, A. T., L. C. NORRIS AND G. F. HEUSER 1931 The occurrence of a pellagra-like syndrome in chicks. Poultry Sci., vol. 10, p. 166.

ROHRMANN, E., G. E. BURGET AND R. J. WILLIAMS 1934 Pantothenic acid content of animal tissues. Proc. Soc. Exp. Biol. Med., vol. 32, p. 433.

SHOURIE, K. L., AND M. SWAMINATHAN 1940 The synthesis of nicotinic acid by rats. Indian J. Med. Research, vol. 27, p. 679.

SNELL, E. E., R. E. EAKIN AND R. J. WILLIAMS 1940 A quantitative test for biotin and observations regarding its occurrence and properties. J. Am. Chem. Soc., vol. 62, p. 175.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. Ind. Eng. Chem. Anal. Ed., vol. 11, p. 346.

SNELL, E. E., F. M. STRONG AND W. H. PETERSON 1937 Growth factors for bacteria. VI. Fractionation and properties of an accessory factor for lactic acid bacteria. Biochem. J., vol. 31, p. 1789.

SNELL, E. E., AND L. D. WRIGHT 1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem., vol. 139, p. 675.

STRONG, F. M, R. E. FEENEY AND A. C. EARLE 1940 Microbiological determination of pantothenic acid. Abstracts, 100th Meeting Am. Chem. Soc., p. 21.

SUOMALAINEN, P. 1939 a Vitamin A and carotene in the hen egg during hatching. Suomen Kemistelehti, vol. 12B, p. 30. Chem. Abst., vol. 34, p. 145.

———— 1939 b Synthesis of ascorbic acid (vitamin C) in the hen egg during hatching. Ann. Acad. Sci. Fennicae, vol. A53, no. 8. Chem. Abst., vol. 34, p. 1724.

WILLIAMS, R. J., J. R. MCMAHAN, A. K. STOUT AND H. K. MITCHELL 1941 An assay method for inositol. Univ. of Texas Pub. (In press.)

WOOLLEY, D. W. 1940 The nature of the anti-alopecia factor. Science, vol. 92, p. 384.

DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION

XXVIII. THE LACTATION-PROMOTING PROPERTIES OF CYSTINE WHEN ADDED TO CASEIN DIETS [1]

BARNETT SURE

Department of Agricultural Chemistry, University of Arkansas, Fayetteville

(Received for publication June 14, 1941)

As early as 1919 results of experiments conducted at the University of Wisconsin and published in 1924 (Sure, '24 a, b) disclosed that diets entirely satisfactory for growth and reproduction may be entirely inadequate for lactation. Later it became apparent (Sure, '27) that the requirements of the vitamin B complex for lactation are much greater than those for growth. That this is true for various components of the vitamin B complex became evident from quantitative studies using pure crystalline thiamine, riboflavin, pyridoxine, and choline (Sure, '40).

For a number of years various nutritional investigators have reported failures with rats, started on experiments from weaning, to rear their young when subsisting on purified diets devoid of natural foods. The extensive literature has been reviewed recently by Folley and associates ('38, '40) and therefore will be referred to only as it may be pertinent to the results of the studies submitted in this and the accompanying paper (no. XXIX of this series).

The preliminary report of Outhouse ('37), that a purified diet containing yeast, cod liver oil and wheat germ oil proved

[1] Research paper no. 699, Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station.

a complete failure for rearing of young whereas such a diet supplemented with cottonseed oil, oats, and lettuce resulted in successful lactation, stimulated the investigation of various fats and oils as possible lactogogues. Consequently, lard, butterfat, hydrogenated cottonseed oil,[2] olive oil, and hydrogenated cottonseed oil in combination with wheat germ oil,

TABLE 1

Percentage composition of rations.

COMPONENT	RATION									
	16	16-a	17	17-a	18	18-a	19	19-a	20	20-a
Casein (purified)[1]	20	20	20	20	20	20	20	20	20	20
Salts no. 185[2]	4		4		4		4		4	
Salts no. 351[3]		4		4		4		4		4
Dried yeast[4]	10	10	10	10	10	10	10	10	10	10
Lard	15	15								
Butterfat			15	15						
Hydrogenated cottonseed oil[5]					15	15			10	10
Olive oil							15	15		
Wheat germ oil									5	5
Starch	51	51	51	51	51	51	51	51	51	51

[1] Commercial casein was obtained from Atterbury Brothers, New York City. This was purified by washing for 10 days with acidulated water and then by several washings with 25% ethanol at room temperature; then dried at 85 to 90°C. By analysis this product contained 13.85% N, or 88.4% protein when Jones' ('31) factor of 6.38 is used. Therefore the rations actually contained 17.7% protein from this casein instead of the 20% intended. Analysis revealed that the yeast contributed 3.7% additional protein making the total come to 21.4%.

[2] McCollum and Simmonds ('18).

[3] Hubbell, Mendel and Wakeman ('37).

[4] Northwestern Yeast Co., Chicago, Ill.

[5] Crisco.

were incorporated to the extent of 15% in the diet, and studies were made of their influence on fertility and lactation. Realizing that a mineral mixture entirely adequate for optimum success for reproduction and lactation had not as yet been found, this study was carried out with two salt mixtures, that of McCollum and Simmonds, no. 185 ('18) and that of Hubbell,

[2] Crisco.

Mendel and Wakeman, no. 351 ('37). In each experiment there were two males and four females, making a total of twenty males and forty females used. The animals were started after weaning, with initial weights ranging from 60 to 80 gm. The

TABLE 2

Lactation-promoting properties of cystine.

RATION NUMBER	NUMBER OF MOTHERS	TOTAL YOUNG BORN	NUMBER OF STILL-BIRTHS	YOUNG GIVEN TO REAR	YOUNG REARED	LACTATION EFFICIENCY INDEX
16						
With cystine	4	40	0	24	24	100
Without cystine	2	19	10	6	0	0
16-a						
With cystine	4	47	0	30	29	97
17						
Without cystine	4	37	0	24	4	17
17-a						
With cystine	3	47	0	30	30	100
Without cystine	1	11	11			
18						
With cystine	2	16	0	11	11	100
Without cystine	2	18	0	12	0	0
18-a						
With cystine	3	39	0	24	22	91
Without cystine	2	43	14	22	0	0
19						
Without cystine	4	89	17	46	10	22
19-a						
With cystine	4	64	0	42	41	98
Without cystine	1	10	0	6	0	0
20						
With cystine	2	47	0	24	22	91
Without cystine	3	47	0	30	11	37
20-a						
With cystine	3	52	0	30	30	100
Without cystine	2	28	0	18	0	0

rations were planned to contain 20% casein,[3] but actually had 21.4% (see footnotes to table 1). Table 1 gives the percentage composition of the diets and the results on fertility and lactation are presented in table 2.

[3] Supplied by Atterbury Brothers, New York City.

Six drops of cod liver oil [4] as a source of vitamins A and D were given daily to each animal during growth and pregnancy, and 10 drops daily to each mother during lactation. Six to 8 drops of wheat germ oil as a source of vitamin E were given daily to each animal during growth and pregnancy, and 10 drops wheat germ oil daily to each mother during lactation.

Early in this investigation it became apparent that, regardless of the character of fats or oils or composition of salts employed, the mothers were failing to nurse their litters satisfactorily during the first 7 to 10 days and frequently during the first 3 days of lactation. At that time the work of Daggs and Lidfeldt ('38) appeared on the effect of sulphydryl compounds on milk production. They reported that the sulphydryl amino acids existing either as cysteine or in the potential form, cystine or methionine, are dietary lactogogues. The lactogenic potency of oxidized or reduced glutathione could be correlated with the respective contents of cystine or cysteine. Daggs ('35) and Daggs and Toumboulian ('35) had previously demonstrated that the lactation-augmenting effect of high protein diets, liver extracts, etc., is due to the extra amount of cystine or glutathione contained in the diet.

Based on Daggs' findings, an intensive effort was made to prevent failure in lactation by supplementation with additional amounts of cystine. The cystine was incorporated in the ration to the extent of 0.2%. This introduced 30 to 40 mg. daily additional cystine based on 15 to 20 gm. daily food intake during lactation. In some experiments the yeast was removed from the ration and 40 mg. cystine incorporated in a 1500 mg. daily yeast dose, as a source of the vitamin B complex. In two cases 20 mg. daily doses were given. Either the cystine administrations in the yeast or the diets containing cystine were given to the mothers when the growth curves of the nursing young reached a plateau or when abnormal gains were being made early in lactation. Success followed every one of thirty-seven trials. Out of 352 young born to mothers that received cystine as a supplement to the 17.7% casein diets,

[4] Mead-Johnson.

there were no stillbirths, and 209 out of 215 allowed to nurse were weaned, which means a lactation efficiency index of 97%. We consider 21 to 24 days to be a normal lactation period on our best stock diets, and only three mothers showed a prolonged lactation period of 27 to 29 days. On the other hand, out of 282 young born to mothers subsisting on the casein diets without provision of supplementary cystine, there were fifty-two stillbirths and only twenty-five out of 164 young allowed to nurse were weaned, or a lactation efficiency index of only 15%. In several cases lactating mothers were entirely successful with first litters when they received supplementary cystine and entirely failed with second litters when they were denied additional cystine administrations.

When the 1938 paper of Daggs and Lidfeldt appeared and it was then decided to add cystine to the diets, a number of females had already failed with their first litters while on diets not fortified with cystine; and, since the purpose of this investigation was to test the lactation stimulating effect of various fats and oils on two different salt mixtures, no systematic distribution of animals on each diet with respect to cystine additions and omissions was possible. It was clear, however, that regardless of the character of fats or oils or the composition of the salt mixture used, cystine was the limiting factor in all of the rations tested.

DISCUSSION

The role of cystine in nutrition was first investigated by Osborne and Mendel ('15). They found that when casein is incorporated in an otherwise adequate diet to the extent of 18%, young rats grow at a rate considered normal at that time. When, however, the proportion of casein was less than 15%, growth was subnormal, and at a level of 9% growth appeared at approximately half the normal rate. The addition of cystine to the ration was followed by accelerated growth of the experimental animals.

Recently Pavcek and Baum ('41) reported that better growth is observed when an 18% casein diet is supplemented

with 75 mg. cystine daily. It is not surprising, therefore, that cystine supplemented our 17.7% casein diet for lactation. Moreover, the beneficial results of cystine in lactation reported in this paper are in agreement with those of Wright and Haag ('39) who found that the lactation-promoting properties of rations containing approximately 9% of alfalfa crude protein are markedly enhanced by 0.4% addition of l-cystine.

In view of the fact that methionine can be converted to cystine (Beach and White, '37; Tarver and Schmidt, '39) and that the presence of cystine does not improve the quality of a ration containing a sufficiency of methionine (Womack, Kemmerer and Rose, '37), similar results in lactation may be anticipated from methionine additions to diets containing about 18% casein as the only source of protein.

The results of this study do not necessarily show the essential nature of cystine but do show that the lactation-promoting properties of a diet containing about 18% casein are markedly improved by the addition of this amino acid. Since casein contains 3.0 to 3.3% methionine, the rations provided about 0.6% of this amino acid, but apparently this amount is insufficient for rearing of young and needs supplementary cystine for successful lactation.

SUMMARY

The purpose of this investigation was to determine the lactation-promoting properties of various fats and oils, such as lard, butterfat, hydrogenated cottonseed oil, olive oil, and wheat germ oil in combination with hydrogenated cottonseed oil, fed at a 15% level on two types of salt mixtures. The rations contained 17.7% purified casein and 3.7% protein derived from the dehydrated bakers' yeast used as a source of the vitamin B complex. The albino rats used in this study were started on the experiments soon after weaning at initial weights ranging from 60 to 80 gm.

Regardless of the nature of the fats or oils, or the composition of the salt mixture, such diets did not meet the demands of lactation. The limiting factor in the rations was

found to be cystine. When the rations were fortified with 0.2% cystine, or daily administration of 20 to 40 mg. cystine to the lactating mothers was begun as failure of lactation became evident, lactation proceeded successfully and the young were weaned.

The results of this study do not necessarily prove the essential nature of cystine, but do show that the lactation-promoting properties of a diet containing about 18% casein are markedly improved by the addition of this amino acid. Since casein contains 3.0 to 3.3% methionine, the rations provided about 0.6% of this amino acid, but apparently this amount is insufficient for rearing of young and supplementary cystine can meet the need for successful lactation.

LITERATURE CITED

BEACH, E. F., AND A. WHITE 1937 Synthesis of cystine by the albino rat. J. Biol. Chem., vol. 127, pp. 87–95.

DAGGS, R. G. 1935 Technic for studying lactation in rats. J. Nutrition, vol. 9, pp. 575–580.

DAGGS, R. G., AND R. L. TOUMBOULIAN 1935 Effect of various dietary principles on lactation in rats. J. Nutrition, vol. 9, pp. 581–593.

DAGGS, R. G., AND V. S. M. LIDFELDT 1938 The effect of the sulphydryl compounds on milk production. J. Nutrition, vol. 15, pp. 211–221.

FOLLEY, S. J., E. W. IKIN, S. K. KON AND H. M. S. WATSON 1938 Observations on specific nutritional factors in lactation. Biochem. J., vol. 32, pp. 1988–2000.

———— 1940 Lactation. Biol. Rev., vol. 15, p. 421.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture in experimental diets. J. Nutrition, vol. 14, pp. 273–286.

JONES, D. B. 1931 Factors for converting percentage of nitrogen in foods and feeds into percentages of proteins. U. S. D. A. Circular No. 183, pp. 1–22.

MCCOLLUM, E. V., AND N. SIMMONDS 1918 A study of the dietary essential, water-soluble B, in relation to solubility and stability towards reagents. J. Biol. Chem., vol. 33, pp. 55–89.

OSBORNE, T. B., AND L. B. MENDEL 1915 The comparative nutritive value of certain proteins in growth and the problem of the protein minimum. J. Biol. Chem., vol. 20, pp. 351–378.

OUTHOUSE, J. 1937 Rep. Ill. Agr. Exp. Sta., 1935–36, p. 285.

PAVCEK, P. L., AND H. M. BAUM 1941 Relation of cystine to achromotrichia. Proc. Soc. Exp. Biol. and Med., vol. 47, pp. 271–272.

SURE, B. 1924 a The existence of a specific vitamin for reproduction. J. Biol. Chem., vol. 58, pp. 693–710.

———— 1924 b The existence of the reproductive dietary complex (vitamin E) in the ethereal extracts of yellow corn, wheat embryo, and hemp seed. J. Biol. Chem., vol. 62, pp. 371–397.

———— 1927 Vitamin B requirements for normal lactation. J. Biol. Chem., vol. 74, pp. 55–70.

———— 1940 Quantitative requirements of the components of the vitamin B complex for lactation and growth of nursing young of the albino rat. J. Nutrition, vol. 19, pp. 57–70.

TARVER, H., AND C. L. A. SCHMIDT 1939 The conversion of methionine to cystine. J. Biol. Chem., vol. 130, pp. 67–81.

WOMACK, M., K. S. KEMMERER AND W. C. ROSE 1937 The relation of cystine and methionine to growth. J. Biol. Chem., vol. 121, pp. 403–411.

WRIGHT, L. D., AND J. R. HAAG 1939 The lactation-promoting effect of l-cystine when fed with alfalfa proteins. J. Nutrition, vol. 17, pp. 263–268.

DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION

XXIX. THE EXISTENCE OF A NEW DIETARY FACTOR ESSENTIAL FOR LACTATION [1]

BARNETT SURE

Department of Agricultural Chemistry, University of Arkansas, Fayetteville

(Received for publication June 14, 1941)

Before the existence of a new dietary factor essential for lactation could be established the quantitative requirements of the various known components of the vitamin B complex in pure form had to be determined. The results of such a study were recently published (Sure, '40). At that time pure pantothenic acid was not available, and the stimulating effect of the "W" factor extracts undoubtedly was due in part to pantothenic acid (Black, Frost and Elvehjem, '40).

It was realized that with the procedure involving transfer of experimental animals from stock to purified diets, during the short period of depletion of the reserves of the vitamin B complex there may be storage of one or more of these dietary essentials. Therefore, an extensive study was made of the fertility and lactation requirements for the various components of the B complex starting with albino rats at weaning, 25 to 28 days of age, and weighing 60 to 80 gm. each. Two males and four females were taken for each experiment. The basal diets and the supplements used are given in table 1.

[1] Research paper no. 721, Journal Series, University of Arkansas. Published with the approval of the Director of the Arkansas Agricultural Experiment Station. Aided by a grant from the Committee on Scientific Research of the American Medical Association.

The females were bred at 80 to 90 days of age when they weighed 160 gm. or over, at which time the daily doses of supplements were increased as indicated in table 1. On the day of birth of the young these daily doses of supplements were again increased and these dosages maintained throughout lactation (table 1). Early in this study the rations contained 15% of hydrogenated cottonseed oil, but with the exception of ration C a change was made later to 10% butterfat,

TABLE 1

Percentage composition of basal rations, and amounts of supplements used.

COMPONENT	BASAL RATION					
	A	B	C	D	E	F
Casein (purified)[1]	20.00		20.00		20.00	10.00
Blood fibrin[2]		20.00		20.00		10.00
Dextrin	60.65	60.95	55.65	60.95	60.75	60.75
Butterfat	10.00	10.00		10.00	10.00	10.00
Crisco			15.00			
Hubbell-Mendel-Wakeman salts no. 351[3]	4.00	4.00	4.00			
Sure's salts no. 1[4]				4.00	4.00	4.00
Wheat germ oil	3.00	3.00	3.00	3.00	3.00	3.00
Agar-agar	2.00	2.00	2.00	2.00	2.00	2.00
Cystine	0.30		0.30		0.20	0.20
Nicotinic acid	0.05	0.05	0.05	0.05	0.05	0.05

SUPPLEMENTS	FROM WEANING TO MATING	DURING PREGNANCY	DURING LACTATION
Thiamine[5]	20 μg.	50 μg.	120 μg.
Riboflavin[5]	20 μg.	50 μg.	120 μg.
Pyridoxine[5]	20 μg.	50 μg.	120 μg.
Choline chloride[5]	6 mg.	9 mg.	15 mg.
"W" extract	0.2 gm.[6]	0.5 gm.[6]	1.0 gm.[6]
Vitamin K[7]	100 μg.	250 μg.	600 μg.
Halibut liver oil	2 drops[8]	2 drops[9]	2 drops[9]

[1] Thoroughly extracted with acidulated water and dilute ethanol.

[2] Thoroughly extracted with 95% ethanol.

[3] Hubbell, Mendel and Wakeman ('37).

[4] Composition given in table 4.

[5] Daily.

[6] Equivalent to this weight of original liver extract.

[7] Given as 2-methyl, 1-4 naphthoquinone.

[8] Once weekly.

[9] Three times weekly.

because of the report by Schantz, Elvehjem and Hart ('40) that butterfat is superior to vegetable oils homogenized into skimmed milk in meeting the nutritive demands of early growth. All rations except B and D, which contained 20% blood fibrin, were fortified with 0.2 to 0.3% cystine from the time of mating.

The experiments may be divided into two series, I in which the Hubbell-Mendel-Wakeman salt mixture no. 351 ('37) was used, and II in which a modification of the Phillips and Hart ('35) salt mixture was employed (referred to as Sure's salts no. 1). The findings of these studies are summarized in tables 2, 3 and 5. Table 4 gives the new salt mixture.

Series I experiments

These experiments were conducted on rations A, B and C which contained the Hubbell-Mendel-Wakeman salt mixture. Ration B used in this series of experiments and ration D used in series II experiments contained blood fibrin [2] (table 1). Beef-blood fibrin was used because of the report by J. H. Jones ('38) that this protein can support good growth at comparatively low levels of supply and also because it contains 3.5% cystine as well as 2.5% methionine (Toennies, '37) and therefore should not require any supplementation with cystine. After thorough extraction with hot 95% ethanol our fibrin preparation had a nitrogen content of 13.5%. Using the factor 5.92 (D. B. Jones, '31) our intended 20% level of supply of protein in the form of this fibrin in rations B and D proved to be 16%.

Experiments were initiated with and without addition of pure crystalline calcium pantothenate (table 2). It will be noted that reproduction was very abnormal, 46% of the young having been born dead; it also appears that supplementation with as much as 250 μg. daily of calcium panto-

[2] Since the work reported in this paper has been completed, Hegsted and associates ('41) have published a modification of the Phillips and Hart salt mixture, increasing the manganese sulfate from 0.7 gm. to 10 gm. and changing the $K_2HPO_4 \cdot 3H_2O$ to the anhydrous salt.

TABLE 2

Series I experiments. Control experiments with and without calcium pantothenate (rations A and B), and responses in lactation to administration of rice bran and liver extracts.

NUMBER OF FEMALES MATED	RATION	DAILY DOSE OF CALCIUM PANTOTHENATE		TOTAL YOUNG BORN	NUMBER OF STILLBIRTHS	NUMBER OF STERILITIES	YOUNG GIVEN MOTHERS TO NURSE	YOUNG REARED	REMARKS
		During pregnancy	During lactation						
		γ.	γ.						
9	A	0	0	38	19	4	15	0	One mother died 2 days after birth of young.
8	A	100	300	41	29	3	12	0	One mother died during parturition with litter in utero.
1	A	100	300	8	0	..	6	6	Litter was failing on 25th day. After receiving 0.5 gm. of rice bran extract daily the mother weaned the litter 9 days later.
8	A	250	600	36	18	2	14	6	
1	A	250	600	9	1	..	6	6	Litter was failing on 12th day of lactation when mother was given 1.0 gm. of rice bran extracts, daily, following which she successfully weaned her young.
6	B	250	600	29	17	2	11	0	
2	B	250	600	15	0	..	12	12	The litters of these two mothers were failing on the 10th and 17th days of lactation respectively. One gram daily of rice bran extract to mothers resulted in weaning of both litters.
1	B	250	600	8	0	..	6	6	When litter was failing on tenth day of lactation, 1.0 gm. daily of a liver extract to mother resulted in successful weaning of young.

thenate during pregnancy and 600 μg. daily during lactation did not prevent marked failures in lactation. In the cases of three mothers failure of lactation was changed to success after the mothers were given separately from the ration and in addition to the various pure crystalline components of the B complex, 1 gm. daily of an extract from rice bran. A daily dose of only 0.5 gm. of this extract was sufficient for one mother. Failure in lactation was changed to success in the rearing of young of another mother by a supplementary daily dose of 1 gm. of liver extracts. Such observations suggested that the rice bran and liver extracts contained a hitherto unrecognized factor essential for lactation.

In order to find the most potent source of the new lactation factor, a biological assay was carried out on brewers' yeast, rice polishings, defatted wheat embryo, and dried grass, as well as the liver extracts. These food products were tested in the absence of calcium pantothenate but in the presence of the "W" factor and vitamin K. At mating there was administered a daily dose of 500 mg. of the various test products which had to be increased to 750 mg., and in some cases to 1500 mg. in order to secure successful weaning of litters (table 3). It will be noted that wherever a natural food product was used, although reproduction was abnormal in many instances, indicated by either sterilities or stillbirths, a large proportion of females in the experiments of every product tested were able to rear their young. We consider 21 to 23 days as a normal lactation period on our best stock diet, but some of the mothers on ration C (table 3) supplemented with the various test products fed at 500 to 1500 mg. daily were able to rear and wean their young only by prolonging the lactation period to 29 to 38 days. Relative to the liver extracts, it should be pointed out that these were administered from weaning up to mating as 0.2 gm. daily doses. At mating the dose was increased to 0.5 gm. daily. In two instances the daily dose had to be further increased to 1000 mg. These were given in the absence of the "W" factor, since the latter was provided in abundance by such extracts.

TABLE 8

Series I experiments. Distribution of the new lactation factor in natural food products used as supplements (ration C).

NUMBER OF FEMALES MATED	FOOD PRODUCT	DAILY DOSE	TOTAL YOUNG BORN	NUMBER OF STILLBIRTHS	NUMBER OF STERILITIES	YOUNG GIVEN MOTHERS TO NURSE	YOUNG REARED	LACTATION EFFICIENCY INDEX	REMARKS
		mg.						%	
6	Brewer's yeast	500 to 750	45	16	0	18	18	100	
6	Rice polishings	500	62	16	0	30	23	77	Had to increase dose to 750 mg. in one case and to 1500 mg. in another.
6	Defatted wheat embryo	500	23	0	3	18	12	66	Lactation abnormal. Had to increase to 1500 mg. for one mother. Another mother died during middle of lactation period.
6	Dried grass	500	51	17	0	29	29	100	Prolonged lactation in case of 3 mothers, i.e., 29, 31, and 35 days, respectively.
6	Liver extracts (fraction D)	500 to 1000	73	12	0	35	32	91	

It will be noted that thirty-two out of thirty-five young were successfully weaned when the liver extracts were used which gave a lactation efficiency index of 91%. The significant point about the results of these experiments is that such food products as brewers' yeast, dried bakers' yeast (Sure, '41), wheat embryo, rice polishings, rice bran, and liver extracts, all potent sources of the vitamin B complex, are effectual in supplementing thiamine, riboflavin, pyridoxine, choline chloride, nicotinic acid, pantothenic acid, and vitamins A, D, E, and K, for rearing of young of the albino rat.

The next problem was to reduce the high incidence of stillbirths. Examination of the Hubbell-Mendel-Wakeman salt

mixture no. 351 ('37) revealed that it does not furnish a sufficiency of manganese, if the reports of Daniels and Everson ('35) and Orent and McCollum ('31) are made the basis of judgment. The demonstration of the indispensability of zinc in the nutrition of the rat (Stirn, Elvehjem and Hart, '35) led Phillips and Hart ('35) to introduce this element in their salt mixture. The Ca:P ratio in the Hubbell-Mendel-Wakeman salt mixture is about 4 to 1, a ratio unsatisfactory for successful lactation according to Cox and Imboden ('36). A new salt mixture was therefore prepared consisting essentially of the Phillips and Hart mixture modified in ways believed to represent the application of available knowledge concerning the need for various inorganic nutrients. The formula finally devised is given in table 4. The product is referred to as "Sure's salts no. 1." [3]

TABLE 4

Composition of Sure's salts no. 1.

	gm.		gm.
NaCl	335.0	KI	1.6
K_2HPO_4 (anhydrous)	645.0	$MnSO_4$ (anhydrous)	7.0
$CaHPO_4 \cdot 2H_2O$	190.0	$CuSO_4$ (anhydrous)	0.4
$MgSO_4$ (anhydrous)	99.0	$Al_2(SO_4)_2K_2SO_4$	0.4
$CaCO_3$	600.0	NaF	0.5
Ferric citrate (powdered)	55.0	$ZnCl_2$	0.5

Series II experiments

This series of experiments were carried out on rations D, E, and F with Sure's salt mixture no. 1. In all of the series II experiments 100 μg. calcium pantothenate were given to each animal daily, 250 μg. daily during pregnancy and 600 μg. daily during lactation. A ration containing 10% of our purified casein and 10% of hot alcohol-extracted beef-blood fibrin was found to promote greater growth than either 20% casein or 20% blood fibrin; hence, the introduction of ration F. During pregnancy and lactation ration F was supplemented with 0.2%

[3] See footnote 2, page 501.

cystine in the diet, in order to insure a sufficiency of sulphydryl compounds for reproduction and milk secretion (Sure, '41). Similarly, the rest of the rations were fortified with cystine only during gestation and lactation.

Comparison of tables 2 and 3 (rations A, B and C) with table 5 (rations D, E and F) shows that there was a marked improvement in reproduction on rations carrying the new salt mixture. Such comparison can only be made between control animals which received the basal diets devoid of any supplements of natural food products or extracts therefrom. Out of thirty-six animals bred on rations A and B, containing the Hubbell-Mendel-Wakeman salt mixture, there were eleven

TABLE 5

Series II experiments. Rice bran extract as a source of the new lactation factor (Rations D, E, and F) and control experiments.

NUMBER OF FEMALES MATED	DAILY DOSE	TOTAL YOUNG BORN	NUMBER OF STILL-BIRTHS	NUMBER OF STERILITIES	YOUNG GIVEN MOTHERS TO NURSE	YOUNG REARED	LACTATION EFFICIENCY INDEX
	gm.						%
8	0.25	61	0	0	45	0	0
1	0.50	8	0	..	6	0	0
1	0.5 to 1.0	12	0	..	6	4	66
9	1.0	80	0	0	52	49	94
1	1.5	13	0	..	6	6	100
20 controls		151	15	2	98	6	6

sterilities and eighty-four stillbirths out of 184 young born (table 2). On the other hand, on rations D, E, and F, containing Sure's salt mixture, out of twenty animals mated there were only two sterilities, and out of 151 young born there were fifteen stillbirths (table 5). In other words, the change to the new salt mixture produced a reduction of stillbirths from 46 to 10% and a reduction of sterilities from 30 to 10%. Lactation was, however, a marked failure even with the new salt mixture. Only one female out of twenty reared her young. Out of ninety-eight young given to rear only six were weaned, or an incidence of infant mortality of 94%. Further supple-

mentation of Sure's salts with cobalt and boron in the form of cobalt chloride and sodium tetraborate (anhydrous), respectively, in the same concentration as the zinc chloride in that salt mixture did not prevent complete failure in lactation. Out of thirty-six young given to six mothers to rear, all died within the first few days of lactation.

It is difficult at present to say what change in the salt mixture is to be credited with the greater success secured in reproduction. We incline to the view that the increase in manganese content was a determining factor. It is also possible that a change in the Ca : P ratio played an important role.

It was anticipated that the administration of 0.25 gm. of the rice bran extract daily during pregnancy would perhaps furnish a sufficiency of the new dietary essential at least during the first week of lactation; from the point of subsequent failure of young, administration of various extracts or concentrates could be begun. Such procedure, however, proved a failure (table 5). Reproduction was excellent. Out of sixty-one young born to eight mothers all were alive and virile but most of the young died on the second day of lactation. A 0.5-gm. dose was tried on only one mother but it proved a failure. When another mother on a 0.5 gm. allowance lost two of her six young on the third day, the daily dose was increased to 1.0 gm. which resulted in the successful rearing and weaning of the rest of the litter. The increases of the rice bran extract to 1.0 gm. daily resulted in the successful rearing of forty-nine out of fifty-two young given nine mothers to rear, or a lactation efficiency index of 94%. One mother reared her litter on a 1.5 gm. daily dose during gestation and lactation. The average lactation period was 23 days which is quite normal according to our experience.

Calculated from the biological assay of the rice bran extract given by the manufacturers of this product, each gram contained 107 μg. thiamine, 7 μg. riboflavin, 107 μg. pyridoxine, and 284 μg. pantothenic acid. Accordingly each female that received a supplementary dose of 1.0 gm. of the rice bran extract, was given the following daily doses of the components of the B complex: 15 μg. thiamine, 110 μg. riboflavin, 15 μg.

pyridoxine, 15 mg. choline chloride, 150 μg. calcium pantothenate and the "W" factor extract from 1.0 gm. liver extracts. It was particularly essential that the thiamine dose be adjusted, because of the possibility of encountering toxic doses for fertility and lactation (Perla, '37; Perla and Sandberg, '39; Sure, '39).

Attempts to save failing nurslings the second or third days of lactation by giving the rice bran extracts or liver extracts were unsuccessful; also attempts to provide the lactation factor later than the seventh day of pregnancy were unsuccessful. The most dependable procedure for insuring successful lactation was to provide a sufficiency of the lactation factor beginning at the time of breeding. Four females on ration F, given 1.0 gm. of liver extracts during pregnancy and lactation, had thirty-seven live young and successfully reared twenty-two out of twenty-three young in an average lactation period of 21 days. Two litters weaned by their mothers in 18 days showed accelerated growth.

Is the new lactation-promoting factor organic or inorganic?

In order to determine whether the lactation factor is organic or inorganic, the rice bran extracts [4] were ashed, and the ash mixed with ten times the amount of sucrose (equivalent to 1.0 gm. of the original extract) was administered daily in caster cups. That the lactation factor is not an inorganic substance became evident from the following results: Eight out of nine mothers failed in lactation. In addition, twelve females failed in lactation when receiving the daily dose of ash from liver extracts [5] equivalent to 1.0 to 2.0 gm. of the original extracts. It is evident then that the lactation factor must be organic in nature.

[4] The ash from the rice bran extracts was prepared by the Research Laboratories of Galen Company at a temperature of about 1000°C. The ash from the liver extracts was prepared by the Research Laboratories of Merck and Company at a temperature approximating 1600°C.

[5] See footnote 4.

Response in lactation to inositol administration

The addition of five to ten times the amount of the "W" factor found necessary for optimum growth did not prevent failure in rearing of young. Furthermore, Frost and Elvehjem ('37) state that the residue from the extract of the "W" factor contained very little activity. After rearing three litters successfully on the residue equivalent to 2 gm. of the original liver extracts a fuller's earth adsorption product was prepared. The new lactation factor was found in the filtrates. On the daily allowance of this concentrate equivalent to 2 gm. of liver extracts containing 320 mg. solids, five mothers reared thirty-three out of thirty-four young. The litter of one mother, however, reached maintenance on the fifteenth day of lactation and maintenance persisted for 7 days. Another litter showed loss of weight on the seventeenth day and maintenance on the succeeding 3 days.

The recent report of Ansbacher ('41) that p-aminobenzoic acid is a chromotrichia factor for the rat, and that of Woolley ('41) that inositol is an antialopecia factor for the mouse, as well as the claim of Pavcek and Baum ('41) that inositol is an "antispectacled" and growth-promoting factor for the rat, warranted trial of these substances for effect on lactation. The results with daily doses of 15 mg. p-aminobenzoic acid were negative. A daily dose of 30 mg. inositol resulted in a prompt response in the case of the first mother, i.e., a gain of 16 gm. in 24 hours and 33 gm. in 48 hours in weights of the litter, and weaning of the litter in 8 days subsequent to the inositol administration. The response to inositol therapy in the case of the second mother was similar.

Influence on lactation of administration of p-aminobenzoic acid, in presence and absence of inositol

It was then decided to attempt to rear nursing young of the albino rat on diets in which the B complex was supplied by only the pure chemical substances proven to date to make up

this complex. For this reason the "W" factor was withheld from the females at mating. The experiments were conducted in three series and the following daily additions to the synthetic vitamin B complex mixture were given to the mothers during pregnancy and lactation: (a) 15 mg. p-aminobenzoic acid; (b) 30 mg. inositol; (c) 15 mg. p-aminobenzoic acid and 30 mg. inositol. The results obtained to date on reproduction and lactation, respectively, are as follows: (a) Out of ninety-two young born there were only three dead, or 3.3% stillbirths; out of fifty-three young given nine mothers to rear, thirty-two were weaned. (b) Out of five litters, two were born dead; two mothers failed in lactation with litters of six each; one mother weaned five young. Per cent of stillbirths was 30. (c) Out of forty-six young born to five mothers there was only one stillbirth; out of twenty-eight young given five mothers to rear, twenty-two were weaned.

Combining the results of series 1 and 3, it is evident that out of eighty-one young allowed to nurse, fifty-four were weaned or a lactation efficiency index of 67% due to the addition of p-aminobenzoic acid, and in the absence of the "W" factor. When it is considered that in the absence of the new lactation factor and in the presence of the "W" factor, the lactation efficiency is only about 5%, the lactation-promoting properties of p-aminobenzoic acid assume marked significance. It would appear then that either p-aminobenzoic acid or a substance of similar physiological properties is a component of the new dietary factor essential for lactation.

During the past 7 years several papers have appeared by Nakahara and associates ('34, '35, '39) on the existence of "L and L_1" factors essential for lactation. Since the diets of the Japanese workers contained natural foods and were deficient in more than one respect, it is impossible to evaluate the results of their findings and to make correlations of their results with those submitted in this communication.

DISCUSSION

It is apparent from the experimental data submitted that an adequacy of the known pure crystalline components of the vitamin B complex, namely, thiamine, riboflavin, pyridoxine, and pantothenic acid, which were fed in amounts six times that required for excellent growth, did not satisfy the requirements for lactation. Choline chloride and nicotinic acid did not supply the missing factor essential for rearing of young. Suspecting that possibly various components of the B complex were being furnished in insufficient amounts during gestation, lactation-doses were given to six mothers beginning at mating, but the failure in lactation was 100%. The daily supplementary dose of the rice bran extract, which changed a 95% failure in lactation to 91% success, introduced only 8.8 mg. nitrogen, of which 1.47 mg. was ammonia, 0.45 mg. urea nitrogen, and 3.0 mg. amino acid nitrogen. It seems then that only 4 to 5 mg. was utilizable nitrogen. In view of the fact that an excellent source of amino acids was furnished by the proteins in rations A to F inclusive, and the amount of nitrogen introduced by the rice bran extract was so minute, it seems improbable that the lactation factor is a known amino acid. On the other hand, the distribution of the factor in such food products as brewers' yeast, bakers' yeast, wheat embryo, rice polishings, rice bran extract, and liver extracts, indicates that it may properly be described as associated with the vitamin B complex. I, therefore, tentatively propose to designate this new lactation factor as "B_x" the "B" associating it with the "B" complex and the "x" denoting that it is as yet chemically unidentified. It is possible, of course, that the "B_x" factor is composed of more than one dietary substance essential for lactation.

The marked responses obtained in lactation following p-aminobenzoic acid additions in the absence of any extracts from natural food products point to the possibility that either this substance or a substance of similar physiological proper-

ties is a component of the "B_x" factor. The response obtained to inositol in lactation also suggests that this substance may be another component of the "B_x" factor.

SUMMARY AND CONCLUSIONS

Young albino rats can exhibit excellent growth on diets in which the vitamin B complex was supplied by pure thiamine, riboflavin, pyridoxine, choline, pantothenic acid, nicotinic acid and "W" factor from liver extracts. Such diets with the amounts of these supplements greatly increased will not support either adequate reproduction or lactation. Experiments with new salt mixtures resulted in marked improvement in reproduction but lactation was still a failure. A rice bran extract and a liver extract were found to supply a factor, the presence of which resulted in 90% success in lactation. Tests with the ash of such products were negative; hence, the factor is organic. Para-aminobenzoic acid or a related compound is a component of the new factor tentatively designated "B_x"; preliminary tests suggest that inositol may also be a component.

The author is indebted to Dr. David Klein of the Wilson Laboratories for supplying fraction D of liver extracts; to Parke, Davis & Co., for the 2-methyl-1-4-napthoquinone; to Armour & Co., for the beef blood fibrin; to Merck & Co., for the thiamine, riboflavin, pyridoxine, calcium pantothenate, and choline chloride; to Mr. H. A. Smith of the Galen Co., Berkeley, Cal., for the rice bran extracts; and to the Cerophyl Laboratories, Kansas City, Mo., for the dried grass.

LITERATURE CITED

ANSBACHER, S. 1941 p-Aminobenzoic acid, a vitamin. Science, vol. 93, pp. 164–165.

BLACK, S., D. V. FROST AND C. A. ELVEHJEM 1940 The relation of vitamin B_6 and pantothenic acid to factor W studies. J. Biol. Chem., vol. 132, pp. 65–76.

COX, W. M., JR., AND M. IMBODEN 1936 The role of calcium and phosphorus in determining reproductive success. J. Nutrition, vol. 11, pp. 147–177.

DANIELS, A. L., AND G. J. EVERSON 1935 The relation of manganese to congenital debility. J. Nutrition, vol. 9, pp. 191–204.

FROST, E. V., AND C. A. ELVEHJEM 1937 Further studies on factor W. J. Biol. Chem., vol. 121, pp. 255–273.

HEGSTED, D. M., R. C. MILLS, C. A. ELVEHJEM AND E. B. HART 1941 Choline in nutrition of chicks. J. Biol. Chem., vol. 138, pp. 459–467.

HUBBELL, R. B., L. B. MENDEL AND A. J. WAKEMAN 1937 A new salt mixture for use in experimental diets. J. Nutrition, vol. 14, pp. 273–286.

JONES, D. B. 1931 Factors for converting percentages of nitrogen in foods and feeds into percentages of proteins. U. S. D. A. Circular, No. 183, pp. 1–22.

JONES, J. H. 1938 The use of fibrin in synthetic diets. J. Nutrition, vol. 15, pp. 269–276.

NAKAHARA, W., F. INUKAI AND S. KATO 1934 A specific dietary factor for lactation. Proc. of Imp. Academy, vol. 10, pp. 268–271.

NAKAHARA, W., F. INUKAI AND D. UGAMI 1935 On the nature of factor L, a specific dietary factor for lactation. Inst. Phys. Chem. Res., vol. 28, pp. 154–161.

———— 1939 Notes on the methods for vitamin L tests. Inst. Phys. and Chem. Res., vol. 36, pp. 327–335.

ORENT, E. R., AND E. V. MCCOLLUM 1931 Effects of deprivation of manganese in the rat. J. Biol. Chem., vol. 92, pp. 651–679.

PAVCEK, P. L., AND H. M. BAUM 1941 Inositol and spectacled eye in rats. Science, vol. 93, p. 502.

PERLA, D. 1937 Toxic effects of an excess of B_1 in rats. Proc. Soc. Exp. Biol. and Med., vol. 37, pp. 169–172.

PERLA, D., AND M. SANDBERG 1939 Metabolic interdependence of vitamin B_1 and manganese. Reciprocal neutralization of their toxic effects. Proc. Soc. Exp. Biol. and Med., vol. 41, pp. 522–527.

PHILLIPS, P. H., AND E. B. HART 1935 The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. J. Biol. Chem., vol. 109, pp. 657–664.

SCHANTZ, E. J., C. A. ELVEHJEM AND E. B. HART 1940 The comparative nutritive value of butterfat and certain vegetable oils. J. Dairy Sci., vol. 23, pp. 181–189.

STIRN, F. E., C. A. ELVEHJEM AND E. B. HART 1935 The indispensability of zinc in the nutrition of the rat. J. Biol. Chem., vol. 109, pp. 347–360.

SURE, B. 1939 Influence of massive doses of vitamin B_1 on fertility and lactation. J. Nutrition, vol. 18, pp. 187–194.

SURE, B. 1940 Quantitative requirements of the components of the vitamin B complex for lactation and growth of nursing young of the albino rat. J. Nutrition, vol. 19, pp. 57–70.

———— 1941 The lactation-promoting properties of cystine when added to casein diets. J. Nutrition, vol. 22, pp. 491–498.

TOENNIES, G. 1937 The sulfur containing amino acid methionine. Growth, vol. 1, pp. 337–370.

WOOLLEY, D. W. 1941 Identification of the mouse antialopecia factor. J. Biol. Chem., vol. 139, pp. 29–35.

THE INFLUENCE OF PRENATAL DIET ON THE MOTHER AND CHILD[1]

J. H. EBBS, F. F. TISDALL AND W. A. SCOTT
Departments of Paediatrics and Obstetrics, University of Toronto, Canada

(Received for publication June 16, 1941)

During the past 25 years, the number of deaths in infants under 1 year of age has been markedly reduced. The number of deaths in the first few weeks of life, however, has been altered very little. Apart from congenital abnormalities, birth trauma and prematurity, there still remain a number of unexplainable deaths. This study was undertaken in order to determine the effect of poor and good prenatal diets upon the outcome of pregnancy and condition of infant during first months of life. Only patients who had not reached the end of the sixth month of pregnancy and those patients who signified their intention of being confined in the Toronto General Hospital were included in the study. If any major disease was found that patient was excluded.

Briefly, the method of study was as follows: (1) An analysis of the patient's food record was made at the beginning of observation; (2) this analysis was repeated 2 months later; (3) patients were classified into three groups, namely, those receiving a poor diet throughout, or having a supplemented diet, or subsisting on a good diet and receiving advice; (4) blood examinations were made for hemoglobin, vitamin C and phosphatase; (5) obstetrical rating was given each patient for (a) the prenatal period, during labor and convalescence, and with respect to (b) condition of baby at birth, (c) follow-up examination of the mother, (d) ability of mother to nurse

[1] Presented at the eighth annual meeting of the American Institute of Nutrition, Chicago, April 16, 1941.

infant, and (e) the whole course of pregnancy from beginning to end of observation; (6) babies were examined at 6 months and at 12 months of age, and records were kept of illnesses, general condition and eruption of teeth.

Each patient at the first interview was given a record form containing a space for each meal for 7 days. In this she was instructed to write down the kind and amount of each food eaten at each meal for 7 days. She was instructed to record weights of food, where possible; otherwise, to record in common measures, such as tablespoonful, cupful, ounces, or measure of solid foods in inches, or as large, medium or small vegetable or fruit. The patient then returned with this record and was interviewed by the dietitian. Each detail on the record was checked by means of questioning and comparison with amounts bought and amounts served at each meal to the whole family. Recipes were also discussed and methods of cooking. Social workers visited the homes of many of the patients in the Supplemented Group in order to check the consumption of the foods being sent. A trained worker visited the home of a small number of patients in each group and weighed the food after it had been estimated and recorded. Having arrived at the approximate amounts consumed, the foods were then totalled for the week under the following headings: eggs, meat (to include fish and fowl), milk, cheese, cream, butter, oil, bread (white and brown), cereals, potatoes, vegetables, cooked and raw, fruits (citrus and others), sugar, and miscellaneous. This list formed the basis for scoring the diet as "good," "fair" or "poor". A consultation was then held and with this diet record, knowledge of the family income, rent, and number of dependents, each patient was considered for further study.

If the diet was poor and the income low, each alternate patient was selected for help from a special fund.[2] Those who did not receive help were left on their poor diets, were given no dietary advice, and will be referred to as the Poor Diet Group. There were 120 in this group. Those who had

[2] This fund was provided by a Toronto business man.

been on an equally poor diet until the fourth or fifth month of pregnancy and then received extra food from us, will be referred to as the Supplemented-to-Good Diet Group. There were ninety of these. It was found that approximately one-half of the patients in the clinic were on moderately good diets and had sufficient income to provide a good diet if given advice. Advice in detail was then given. These will be referred to as the Good Diet Group. There were 170 of these studied.

As a basis for planning reasonable food requirements during pregnancy, we aimed at the following amounts of the essential foods: daily, 40 ounces of milk, 1 ounce cheese, 1 egg, an average serving of butter and meat, 2 servings of vegetables in addition to potato, one orange or one-half grapefruit, or 5 ounces of tomato juice, one-half of the cereals and bread consumed to be in whole grain form, 2 teaspoonfuls of cod liver oil or equivalent in concentrate, liver once a week, salt to be iodized, and medicinal iron to be used if indicated. Two tablespoonfuls of wheat germ daily were advised. The constitution of such an average diet is given in table 1.

TABLE 1

Nutritive factors yielded by the recommended Good Diet used in this study.

		Vitamins:	
Calories	— 2400–2800	A	— 6000 I.U.[1]
Protein	— 80–100 gm.	B_1	— 500–1000 I.U.
Fat	— 80–100 gm.	B_2	— 3.0–3.5 mg.
Carbohydrate	— 350–400 gm.	C	— 50–75 mg.
Calcium	— 1.5 gm.	D	— 500–1000 I.U.
Iron	— 0.020 gm.		
Iodine	— In iodized salt	[1] International Units.	

We advised those patients with sufficient income in the Good Diet Group to try to obtain the amounts given in table 1.

To the patients in the Supplemented-to-Good Group we sent daily, 30 ounces of milk, 1 egg and 1 orange. Once a week, we also sent two 16-ounce tins of factory canned tomatoes and ½ pound of cheddar cheese. At the clinic we distributed a palatable dried wheat germ which contained malt, and added

iron.[3] Two tablespoonfuls contained 12 mg. of iron. Viosterol capsules containing 2000 international units of vitamin D were supplied, with instructions to take one daily.[4] Advice in detail was given to the women in this group regarding the use of this extra food, and instruction was given in planning the remainder of the diet from the family income.

The average cost of the extra food supplied to the ninety women in the Supplemented-to-Good Diet Group for an average period of 4.7 months was $25.00 per patient.

In order to offset any possible psychological factor due to the taking of medicine, patients not receiving supplemental food were given gelatin capsules resembling the viosterol capsules, but containing instead plain corn oil.

The additional food supplied to the patients in the Supplemented-to-Good Group gave the following daily average values: protein, 45 gm.; fat, 46 gm.; carbohydrate, 60 gm.; calories, 840; calcium, 1.45 gm.; iron, 15 mg.; vitamin C, 50–80 mg.; vitamin B_1, 350–400 I.U., and vitamin D, 2000 I.U.

In order to eliminate errors in judgment and to offset the number of dietitians interviewing the patients, each diet record was later calculated for protein, fat, carbohydrate, calories, calcium and iron.[5] The material was arranged in such a form that information regarding the vitamin content could also be calculated.

In table 2, it will be noted that the Poor Diet Group and the Supplemented Group were equally low in every respect in the first record made at the beginning of the observation. The average figures of the diets of patients in the Good Diet Group are moderately good. In the second record, made about 4 or 6 weeks before confinement, the average of the diets in the Poor Diet Group is still low, although slightly increased over the first record. It will be noted that the figures in the Supplemented Group and the Good Diet Group have improved

[3] Embryon—Scientific Foods, Ltd., Toronto.

[4] Kindly supplied by Mead Johnson & Company.

[5] Figures for calculation of protein, fat, carbohydrate, calories, calcium and iron were chosen mainly from table 13 of "Applied Dietetics" by Francis Stern.

TABLE 2

Analysis of diets in pregnancy.[1]

		PROTEIN	FAT	CH	CALS.	Ca	Fe
		gm.	*gm.*	*gm.*		*gm.*	*mg.*
First record	Poor Diet	56	66	213	1672	.537	10.7
	Supplemented-Good Diet	56	67	212	1690	.562	10.5
	Good Diet	81	95	261	2206	.886	14.2
Second record	Poor Diet	62	75	232	1837	.746	11.6
	Supplemented-Good Diet	94	111	283	2424	1.61	24.3
	Good Diet	92	113	293	2521	1.30	18.3

[1] All figures are per diem.

markedly, the former by supplying food, and the latter by education.

Table 3 shows the percentage of patients in each group on the basis of their protein intake. The improvement in the Supplemented and the Good Diet Groups will be noted. Sixty per cent of the patients in the Poor Diet Group were taking less than 60 gm. of protein daily. Seventy-eight per cent in the Supplemented Group were getting more than 80 gm. daily after the extra food was supplied. Advice to those in the Good Diet Group about increasing the consumption of milk,

TABLE 3

Percentage of patients in each group on basis of daily consumption of protein, calcium and vitamin C.

	POOR DIET		SUPPLEMENTED-GOOD		GOOD DIET	
	1st record	2nd record	1st record	2nd record	1st record	2nd record
	%	%	%	%	%	%
Protein						
Less than 60 gm.	60	38	54	2	23	2
60 to 80 gm.	33	48	41	20	40	24
More than 80 gm.	7	14	5	78	37	74
Calcium						
Less than 0.8 gm.	81	61	86	2	46	2
0.8 to 1.2 gm.	16	28	10	10	32	37
More than 1.2 gm.	3	11	4	88	22	61
Vitamin C						
Less than 25 mg.	82	69	84	2	46	34
25 to 50 mg.	17	31	15	48	46	56
More than 50 mg.	1	0	1	50	8	10

eggs, meat and cheese resulted in a substantial increase in the percentage who were taking a reasonable amount of protein. Thirty-six per cent of the Poor Diet Group were getting less than 0.4 gm. of calcium daily. Eighty-six per cent of the patients in the Supplemented Diet Group were getting less than 0.8 gm. of calcium daily, according to our estimated figures in the first record. The addition of milk and cheese to the diet of the Supplemented Group improved the second record greatly. All but 2% were getting more than 0.8 gm. Again the effect of education was noted in the Good Diet Group, in which all but 2% brought the intake of calcium above 0.8 gm. daily. The figures for vitamin C are based only upon the consumption of citrus fruits and tomatoes. They do not include any source of vitamin C in the general diet. It will be noted that the economic level of even the Good Diet Group did not allow the women to purchase more than 50 mg. of vitamin C in citrus fruits or tomatoes.

Table 4 illustrates how the poor diet in the Supplemented Group was improved as far as vitamin B_1 was concerned, by

TABLE 4

Vitamin B_1 content of low income diets.

		INT. UNITS OF VITAMIN B_1
Original diet —		
8 oz. potatoes		100
8 oz. bread — 80% white		54
1.5 oz. cooked oatmeal (7.5 gm. dry)		13
10 oz. milk		60
2.7 oz. meat (beef six times, pork once)		67
4 oz. vegetable		23
0.5 oz. egg — ½ egg		7
		324
Supplemented by —		
0.5 oz. wheat germ	100	
30 oz. milk	180	
1 egg	30	
1 orange	20	
4.5 oz. tomato	34	
Additions to meat, vegetables and whole wheat bread	*86*	*450*
TOTAL		*774*

giving wheat germ, milk, egg, orange and tomato, and by changing from white bread to whole wheat bread.

The average duration of observation in the Prenatal Clinic was 4.4 months in the Poor Diet Group, 4.7 months in the Supplemented Group and 4.4 months in the Good Diet Group (table 5). The economic status of the patients in the three diet groups is shown in table 5. Three per cent of the patients in the Good Diet Group were receiving from other sources extra milk, meat, vegetables and fruits in addition to the relief ration, which allowed them to be in the group improved by education. The past obstetrical records of the multiparous

TABLE 5

Patients in prenatal diet study—miscellaneous information.

	POOR DIET	SUPPLEMENTED-GOOD DIET	GOOD DIET
Number of patients	120	90	170
Average age	26	27	25
Average duration of prenatal observation	4.4 mos.	4.7 mos.	4.4 mos.
Primipara	31%	29%	46%
Percentage of families on welfare relief	44%	48%	3%
Average value of relief allowance (approximate only)	$7.50	$8.50	—
Average weekly income of families not on relief	$12.02	$10.94	$16.94
Average number of persons per family	3.0	3.7	2.8
Average income per person	$3.34	$2.64	$6.02

patients showed a much higher incidence of previous major complications in the Poor Diet and Supplemented Groups than in the Good Diet Group (table 6.) Those in the Supplemented Diet Group had experienced more miscarriages, stillbirths and premature births in previous pregnancies than the Poor Diet Group.

The obstetrician in charge of the patients in the Prenatal Clinic and in the Hospital has given his rating of the condition and progress of the patient in each period of pregnancy. He was unaware of the diet group to which each patient belonged. A "good" or "fair" rating indicated that in his opinion the patient had progressed satisfactorily or with minor compli-

TABLE 6

Observations related to pregnancy.

		POOR DIET	SUPPLEMENTED-GOOD DIET	GOOD DIET
Past obstetrical history of multiparous patients (% of cases):				
Abortions		13.1	4.7	9.0
Miscarriages		38.1	39.0	24.4
Prematures		10.7	20.3	13.3
Stillbirths		9.5	4.7	2.2
Obstetrician's rating during pregnancy (% of cases):				
Prenatal period	Good–Fair	64	91	88
	Poor–Bad	36	9	12
Labor	Good–Fair	76	97	94
	Poor–Bad	24	3	6
Convalescence	Good–Fair	88	96	91
	Poor–Bad	12	4	9
Whole course of pregnancy	Good–Fair	66	94	85
	Poor–Bad	34	6	15
Complications during pregnancy (% of cases):				
Anemia		28.6	16.1	21.6
Preeclampsia		5.0	5.7	4.8
Toxemia		7.6	3.4	3.0
Hemorrhage—prenatal		5.9	5.7	2.4
Threatened miscarriage		8.4	1.1	2.4
Miscarriage		6.0	0	1.2
Premature birth		8.0	2.2	3.0
Stillbirth		3.4	0	0.6
Hemorrhage (during labor)		11.2	10.3	7.7
Endometritis		9.0	3.4	6.1
Mastitis		4.5	2.3	4.8
Breast abscess		3.0	1.1	2.0
Studies of blood during pregnancy (all values are averages):				
Hemoglobin at term		11.5 gm.	12.1 gm.	11.9 gm.
Ascorbic acid at term		0.47 mg. %	0.73 mg. %	0.62 mg. %
Ascorbic acid cord blood		1.0 mg. %	1.4 mg. %	1.3 mg. %
Phosphatase at term		16.5 units	14.5 units	16.6 units

cations only. "Poor" or "bad" meant that many or major complications had arisen. The rating during the prenatal period, during the actual labor, and during the 2 weeks of convalescence in the hospital is shown in table 6. The obstetrician's rating of the whole course of pregnancy from the time that the patient first came under observation in the Prenatal Clinic until she was seen 6 weeks after the birth of her baby is also presented in table 6. The mothers in the Supplemented-to-Good Diet Group proved to be better obstetrical risks. The average duration of labor was 5 hours shorter in this group than in the Poor Diet Group. We noted a marked improvement in the general mental attitude of the patients in the Supplemented Group; many of them lost their minor aches and pains, and no longer had numerous complaints.

During the prenatal period there were more cases of anemia, toxemia, and threatened miscarriage in the Poor Diet Group, while the total number of complications in this group was almost double that in the Supplemented-Good Group (table 6). The complications which affected the rating during labor in the Poor Diet Group were chiefly 6% of miscarriages, 8% of premature births, and 3.4% of stillbirths, while in the Supplemented Good Group there were only 2.2% of prematures and no miscarriages or stillbirths (table 6). After delivery there were fewer cases of uterine or breast infections in the Supplemented-Good Group (table 6).

The effect of prenatal diet is reflected in the average levels of hemoglobin, vitamin C and phosphatase in the blood of the mother at the time of delivery (table 6). The average amount of hemoglobin at the time of delivery was slightly higher in the Supplemented-Good Group. The average level of ascorbic acid in the mother's blood at term and in the cord blood was proportional to the vitamin C obtained by consumption of citrus fruit and tomatoes. Phosphatase is an enzyme which has to do with the laying down of new bone. Phosphatase is increased when there are difficult or abnormal conditions in bone formation. Thus we have found that the phosphatase of the mother's blood is more than double the average values

from the sixth month to term when twins are present (Ebbs and Scott, '40). The phosphatase in the mother's blood was appreciably lower in the Supplemented Group than in the other two groups. This became apparent from the seventh month onward after the Supplemented Group had been receiving viosterol capsules, while the other two groups had not received a source of vitamin D. This seemed to indicate that expectant mothers receiving vitamin D and an adequate diet were better able to provide for new bone in the developing fetus.

The average birth weight of the babies born of mothers in the Poor Diet Group was 7 pounds 10 ounces; in the Supplemented Group, 7 pounds 7 ounces; and in the Good Diet Group 7 pounds 6½ ounces. The additional calories do not appear to have influenced the size of the baby.

The relation of prenatal diet to the incidence of breast feeding is shown in table 7.

TABLE 7

Observations related to infancy.

CATEGORIES OF INTEREST		POOR DIET	SUPPLEMENTED-GOOD DIET	GOOD DIET
Breast feeding in relation to prenatal diet (% of cases):				
In hospital	Breast feeding	81	95	88
	Artificial feeding	19	5	12
Six weeks after birth	Breast feeding	59	86	71
	Artificial feeding	41	14	29
Principal illnesses in babies during first 6 months (% of cases):				
Frequent colds		21.0	4.7	4.7
Bronchitis		4.2	1.5	5.7
Pneumonia		5.5	1.5	0.9
Rickets		5.5	0	0.9
Tetany		4.2	0	0
Dystrophy		7.0	1.5	0
Anemia		25.0	9.4	17.1
Deaths		3	0	0

An attempt is being made to follow the progress of the babies born of the mothers in this study to determine the influence, if any, of prenatal diet upon the future condition of the baby. These observations are not completed, but a brief summary can be given of the first 250 babies followed to the age of 6 months (table 7). The increased incidence of minor and major diseases in the babies born of mothers in the Poor Diet Group is quite striking. The general condition of the babies in the Supplemented and Good Diet Groups was on the whole much better. In a large proportion one could tell the diet group of the mother by looking at her baby. Two of the three infant deaths in the Poor Diet Group resulted from pneumonia, and the other from prematurity.

SUMMARY

The prenatal diets of 400 women with low incomes were studied. One group found to be on a poor diet was left as a control, a second group on a poor diet was improved by supplying food during the last 3 or 4 months of pregnancy, and a third group, found to have moderately good prenatal diets was improved by education alone.

During the whole course of pregnancy the mothers on a good or supplemented diet enjoyed better health, had fewer complications and proved to be better obstetrical risks than those left on poor prenatal diets.

The incidence of miscarriages, stillbirths and premature births in the women on poor diets was much increased.

The incidence of illness in the babies up to the age of 6 months and the number of deaths resulting from these illnesses were many times greater in the Poor Diet Group.

CONCLUSION

While it is recognized that there are other important factors in the successful outcome of pregnancy, this study suggests that the nutrition of the mother during the prenatal period

influences to a considerable degree the whole course of pregnancy, and in addition directly affects the health of the child during the first 6 months of life.

ACKNOWLEDGMENTS

We wish to thank the many persons who have helped in this study. Particularly we are indebted to Miss Marjorie Bell, Miss Helen Kelly, Miss Elizabeth Castle, Miss Nina Johnstone, and the obstetrical staff and nursing staff of the Toronto General Hospital.

The diet analyses and diet instruction were personally supervised by Miss Winnifred Moyle, with the assistance of her dietetic staff at the Toronto General Hospital.

LITERATURE CITED

EBBS, J. H., AND W. A. SCOTT 1940 Blood phosphatase in pregnancy an indication of twins. Am. J. Obstetrics & Gynecology, vol. 39, pp. 1043–1044.

THIAMINE, NICOTINIC ACID, RIBOFLAVIN AND PANTOTHENIC ACID IN RYE AND ITS MILLED PRODUCTS

AARON J. IHDE AND HENRY A. SCHUETTE
Department of Chemistry, University of Wisconsin, Madison

(Received for publication July 1, 1941)

It has been generally agreed that the cereal grains, especially the germ and bran portions, are rich sources of some of the vitamins of the B complex. In the case of wheat, considerable data have been presented to show that this is true; data for the other cereal grains are meager. Although rye flour ranks in importance second only to wheat flour, little is known regarding its vitamin content, and even less regarding the distribution of the vitamins throughout the kernel.

Baker and Wright ('35), using a bradycardia technique, reported 22 μg. per gram of thiamine in rye germ. Scheunert and Schieblich ('37), using a rat-feeding method, found 9 μg. per gram of thiamine in rye germ and 3 μg. per gram in the whole rye. Booher and Hartzler ('39), also using a rat growth technique, reported 3.9 and 4.7 μg. per gram of thiamine in two samples of whole rye. Lunde, Kringstad and Olsen ('39) reported 4.2 μg. of thiamine in whole rye meal and 1.6 μg. per gram in rye flour as determined by the thiochrome method. Schultz, Atkin and Frey ('41) tested ten samples of rye for thiamine by their fermentation method ('37) and found a range of 4.0–5.7 μg. per gram with 4.84 as an average figure.

Scheunert and Schieblich ('37), using the Bourquin-Sherman method ('31), found 2.4 μg. per gram of riboflavin in the whole rye and 10 μg. per gram in rye germ. Lunde, Kringstad and Olsen ('39) reported 10 μg. per gram in rye germ. Lunde,

Kringstad and Olsen ('39) reported 1.6 μg. per gram of riboflavin in whole rye meal and 0.6 μg. per gram in rye flour of 60% extraction, as determined by a fluorescence method. Kringstad and Naess ('39), using a cyanogen bromide-aniline method, reported 13 μg. per gram of nicotinic acid in whole rye. Recently Snell and Wright ('41) have reported 58 to 63 μg. per gram in a sample of rye flour as determined by their microbiological method. No assays for pantothenic acid or vitamin B_6 (pyridoxine) are reported to date.

EXPERIMENTAL PART

Analyses for thiamine, nicotinic acid, riboflavin and pantothenic acid were made on representative products[1] of the rye-milling process, namely, whole rye meal, rye germ, middlings, dark flour, and white flour. The middlings and flours were obtained in duplicate series, one ground from whole rye, the other from rye which had been degerminated. The latter series was representative of common milling practice, since the germ is usually removed by a so-called "smutting" process in order to improve the keeping quality of the flour. The white flour samples represent a separation of the straight flour in such manner that the coarse, colored particles are diverted into a dark flour, leaving the "white" fraction largely free of the outer, colored portions of the kernel. Two samples of white flour from whole rye differed in that one was bleached while the other was not so treated. The sample of white flour from degerminated rye was also bleached.

Preliminary assays for thiamine were made by the colorimetric method of Melnick and Field ('39) as modified by Emmett, Peacock and Brown ('40). Satisfactory results were obtained for rye germ (9.3 μg. per gram) and for middlings (3.4 μg. per gram), but concentration difficulties led to the abandonment of the procedure in favor of the method of Henry ('41) which utilizes the growth-response of Phycomyces blakesleeanus to thiamine and its phosphorylated esters as a

[1] Acknowledgment is made to Frank H. Blodgett, Inc., of Janesville, Wisconsin, who furnished the materials used in this investigation.

method for measuring the small amounts present in plant tissues.

The chemical methods for nicotinic acid, based on color-formation with cyanogen bromide and an aromatic amine, have been shown by Kodicek ('40) and Waisman and Elvehjem ('41) to give erroneous results with cereal products. Therefore, the microbiological method of Snell and Wright ('41), based upon lactic acid production by Lactobacillus arabinosus 17-5, was used.[2]

Riboflavin was determined by the microbiological method of Snell and Strong ('39) using Lactobacillus casei ε. Pantothenic acid was determined by the method of Pennington, Snell and Williams ('40) using the same organism.

TABLE 1

Thiamine, nicotinic acid, riboflavin and pantothenic acid in rye products.

PRODUCT	THIAMINE	NICOTINIC ACID	RIBOFLAVIN	PANTOTHENIC ACID
	μg./g.	*μg./g.*	*μg./g.*	*μg./g.*
Whole rye	2.4	12.9	1.5	10.4
Rye germ	9.3	27.0	4.46	13.9
Middlings from whole rye	3.3	16.7	2.5	23.1
Middlings from degerminated rye	3.3	17.7	2.0	23.1
Dark flour from whole rye	3.2	12.2	1.7	13.4
Dark flour from degerminated rye	3.6	12.5	1.8	14.9
White flour from whole rye (not bleached)	1.5	7.1	0.76	7.1
White flour from whole rye (bleached)	1.6	7.3	0.69	7.25
White flour from degerminated rye (bleached)	1.4	7.3	0.68	6.5

In all cases the assays were made on no less than three individual samples of differing weight. In case of doubtful results the assays were repeated until consistent results were obtained. Standard curves were prepared with every set of assays. Recovery experiments were also made on every run with recoveries usually ranging between 90 and 105%. The results obtained, expressed in micrograms per gram, are summarized in table 1.

[2] We wish to thank Prof. F. M. Strong of the Biochemistry Department of the College of Agriculture for his interest and suggestions and Mr. Lester Teply of his laboratory for making the nicotinic acid determinations.

DISCUSSION

The data in table 1 have been averaged and treated in table 2 so as to show what percentages of thiamine, nicotinic acid, riboflavin, and pantothenic acid in the whole grain are diverted into the various products of the milling process.

Although the germ contains nearly four times as much thiamine as the whole grain, it contributes only 6% of the total thiamine of the grain since it constitutes such a small proportion of the kernel.[3] The middlings, representing in large part the covering of the seed, contain one-fourth of the

TABLE 2

Distribution of thiamine, nicotinic acid, riboflavin and pantothenic acid in rye products.

	RYE	THIAMINE	NICOTINIC ACID	RIBOFLAVIN	PANTOTHENIC ACID
	%	%	%	%	%
Whole rye	100				
Germ	1.6	6.1	4.1	5.5	1.9
Middlings	18	24.6	29.2	31.1	35.4
Flour, straight	80.4	69.2	66.7	63.4	62.7
Flour, dark	24.4	34.4	28.8	32.8	29.3
Flour, white	56	34.8	38.0	30.5	33.4

total thiamine while the straight flour contains approximately two-thirds. Further refining of the straight flour results in a concentration of one-half of this thiamine in the dark portion which constitutes only one-third of the total flour.

In spite of this refining loss, white rye flour appears to be a richer source of thiamine than white wheat flour. The value of 1.5 μg. per gram for straight wheat flour reported by Schultz, Atkin and Frey ('39) is equal to the value found for white rye flour during this study. All other recent investigators, however, report lower values for wheat flour. Booher and Hartzler ('39) reported 0.92 μg. per gram, using a rat growth method. Scheunert and Schieblich ('37) report a

[3] Experimental dissections and weighings were made on four different samples of rye grain. The germ content varied between 1.38 and 1.78% with 1.61% as an average figure.

value of 1 μg. per gram, using a similar method. Lunde, Kringstad and Olsen ('39), using a thiochrome method, found 0.9 μg. per gram in straight wheat flour as compared to 1.6 μg. per gram in straight rye flour. Values reported for patent wheat flour are even lower, ranging from 0.5 μg. per gram by Booher and Hartzler ('39) to 0.7 μg. per gram by Schultz, Atkin and Frey ('39).

The lower refining losses in rye flour as compared to wheat flour are further borne out by comparison with the data of Schultz, Atkin and Frey ('39). These workers showed that the straight flour, representing 72% of the wheat kernel, contains only 18% of thiamine present in the original wheat.

It is thus evident that differences in the structure of the rye kernel and in the technique of rye-milling result in a flour nutritionally superior to straight wheat flour insofar as thiamine is concerned. It must also be remembered that white rye flour is seldom marketed as such but is blended with varying amounts of the dark rye flour which further serves to enrich the product.

The distribution of nicotinic acid and riboflavin roughly parallels that of thiamine although rye, like the other cereal grains, cannot be considered a rich source of these vitamins. Pantothenic acid does not appear to be concentrated to any degree in the germ portion, but the middlings are a rich source and the dark flour contains a significant amount.

Reference to table 1 shows that there is no significant difference between the products milled from whole rye and from degerminated rye. The germ constitutes such a small portion of the whole kernel that its removal prior to milling results in only a slight loss of the total vitamins.

Bleaching of the white flour appears to result in no destruction of vitamins except possibly in the case of riboflavin. Slightly lower values for this vitamin were always noticed in bleached samples as compared to unbleached ones. The differences, however, were hardly great enough to be considered conclusive.

SUMMARY

Samples of the products of the rye-milling process were assayed for thiamine, nicotinic acid, riboflavin and pantothenic acid by microbiological methods.

Except for pantothenic acid, the concentration of each of the vitamins is greatest in the germ. The middlings are also a potent source of each of the vitamins but particularly of pantothenic acid.

The flour contains approximately two-thirds of the total vitamins in the whole grain. It is, therefore, a definitely richer source of the vitamin B complex than white wheat flour. Even a high degree of refining fails to bring the thiamine content to the low level characteristic of patent wheat flour.

Rye flour contains approximately one-half of its total vitamins in the dark portion which constitutes only one-third of the total flour.

Degermination of the rye prior to milling fails to remove enough of the vitamins to be detectable by the methods used since the germ constitutes such a small portion of the whole kernel.

Bleaching appears to cause no vitamin losses except possibly in the case of riboflavin.

LITERATURE CITED

BAKER, AUDREY Z., AND MARGARET D. WRIGHT 1935 The vitamin B_1 content of foods. Biochem. J., vol. 29, p. 1802.

BOOHER, LELA E., AND EVA R. HARTZLER 1939 The vitamin B_1 content of foods in terms of crystalline thiamine. U. S. Dept. Agr., Tech. Bull., 707.

BOURQUIN, ANNE, AND H. C. SHERMAN 1931 Quantitative determination of vitamin G (B_2). J. Am. Chem. Soc., vol. 53, p. 3501.

EMMETT, A. D., GAIL PEACOCK AND R. A. BROWN 1940 Chemical determination of thiamine by a modification of the Melnick-Field method. J. Biol. Chem., vol. 135, p. 131.

HENRY, B. W. 1941 The relation of vitamin B_1 to crown-gall development. Ph.D. thesis, University of Wisconsin.

KODICEK, E. 1940 Estimation of nicotinic acid in animal tissues, blood and certain foodstuffs. I. Method. II. Applications. Biochem. J., vol. 34, pp. 712, 724.

KRINGSTAD, H., AND T. NAESS 1939 Eine colorimetrische Methode zur Bestimmung von Nicotinsäure und Nicotinsäureamide in Nahrungsmitteln. Z. physiol. Chem., vol. 260, p. 108.

LUNDE, G., H. KRINGSTAD AND A. OLSEN 1939 The amount of vitamin B_1 and B_2 (riboflavin) in ordinary Norwegian cereals and bread. Nord. Med., vol. 3, p. 2533; Chem. Abst., vol. 34, p. 531 (1940).

MELNICK, D., AND H. FIELD, JR. 1939 Chemical determination of vitamin B_1. I. Reaction between thiamine in pure aqueous solution and diazotized *p*-amino acetophenone. II. Method for estimation of the thiamine content of biological materials with the diazotized *p*-amino acetophenone reagent. III. Quantitative enzymic conversion of cocarboxylase (thiamine pyrophosphate) to the free vitamin. J. Biol. Chem., vol. 127, p. 505; p. 515; p. 531.

PENNINGTON, D., E. E. SNELL AND R. J. WILLIAMS 1940 An assay method for pantothenic acid. J. Biol. Chem., vol. 135, p. 213.

SCHEUNERT, A., AND M. SCHIEBLICH 1937 Über den Vitamingehalt von Weizen und Roggen und der daraus hergestellten Mehle und Brot. Biochem. Z., vol. 290, p. 398.

SCHULTZ, A. S., L. ATKIN AND C. N. FREY 1937 A fermentation test for vitamin B_1. J. Am. Chem. Soc., vol. 59, p. 2457.

———— 1939 The vitamin B_1 content of wheat, flour and bread. Cereal Chem., vol. 16, p. 643.

———— 1941 A preliminary survey of the vitamin B_1 content of American cereals. Cereal Chem., vol. 18, p. 106.

SNELL, E. E., AND F. M. STRONG 1939 A microbiological assay for riboflavin. Ind. Eng. Chem., Anal. Ed., vol. 11, p. 346.

SNELL, E. E., AND L. D. WRIGHT 1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem., vol. 139, p. 675.

WAISMAN, H. A., AND C. A. ELVEHJEM 1941 Chemical estimation of nicotinic acid and vitamin B_6. Ind. Eng. Chem., Anal. Ed., vol. 13, p. 221.

NICOTINIC ACID CONTENT OF MEAT AND MEAT PRODUCTS [1]

J. M. McINTIRE, HARRY A. WAISMAN, LAVELL M. HENDERSON AND C. A. ELVEHJEM

Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison

(Received for publication August 4, 1941)

The nicotinic acid potency of a number of animal tissues was reported in a previous paper (Waisman et al., '40). The results were based on the growth responses in dogs maintained on the modified Goldberger diet. By careful standardization of the animal with the pure vitamin it was possible to obtain a fairly reliable figure for the antipellagra activity of the various tissues. At the time these determinations were made certain limitations in this method were recognized, but it was decided that the animal assay was more reliable than the chemical methods which were available at that time. Much time has been spent during the past 2 years in perfecting the cyanogen bromide method in order to apply it to animal tissue assays. The reports of Melnick and Field ('40), Kodicek ('40), and Bandier ('40) have been especially valuable in helping to perfect our procedure.

EXPERIMENTAL AND RESULTS

The dried meats which were available for the chemical determination of nicotinic acid were those previously assayed for nicotinic acid by the biological method (Waisman et al., '40),

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

Supported in part by a grant from the National Live Stock and Meat Board made through the National Research Council.

as well as additional samples which were assayed previously for thiamine (Mickelsen et al., '39 a), riboflavin (Mickelsen et al., '39 b), pantothenic acid (Waisman et al., '39) and pyridoxine (Henderson et al., '41). The procedure used was essentially that of Melnick and Field ('40) with the slight modifications described by Waisman and Elvehjem ('41 a). In the majority of cases the recovery of the added nicotinic acid was in the range of 90 to 110%.

The results are summarized in table 1. The figures represent the results of over two hundred analyses on seventy different samples. These samples are listed in the approximate order of potency.

The organ tissues, liver and kidney, were found to be the most potent sources of nicotinic acid. The livers of beef, pork, and lamb have the same range of potency, namely, 15.1 to 22.7 mg. per 100 gm. of fresh tissue. This range compares favorably with the 18.0 mg. given by Kringstad and Naess ('38) and the figures of Karrer and Keller ('39) namely 11.8 and 9.3 mg. per 100 gm. of fresh tissue, respectively. Unlike liver, the kidney tissues of beef and pork differ to some extent in nicotinic acid content. The range for beef kidney was 6.4 to 8.3 mg., which is similar to that given by Bandier ('39), but it is considerably lower than the figure of 19.4 mg. per 100 gm. of fresh tissue given by Karrer and Keller. The range of pork kidney was found to be 9.0 to 10.5 mg., which is higher than the figure of 6.5 mg. per 100 gm. of fresh tissue given by Bandier for kidney. Values obtained from assaying the muscular tissue of various animals were in good agreement with one another ranging from 5.6 to 9.1 mg. This range is somewhat higher than the figures reported by Bandier ('39), Kringstad and Naess ('38), and Karrer and Keller ('39) who gave values of 4.73, 4.9 and 3.8 mg. per 100 gm. of fresh muscle tissue, respectively. Our assays on heart, spleen and lung give somewhat higher values than those reported by the other workers. The nicotinic acid content of light and dark chicken meat was found to be about the same. This is interesting since the assays of thiamine, riboflavin, and pantothenic acid have

TABLE 1

Nicotinic acid values obtained by chemical analyses of animal tissues.

TISSUE	SAMPLE NUMBER	MILLIGRAMS OF NICOTINIC ACID PER 100 GM. OF TISSUE	
		Dry weight	Fresh weight
Beef liver	98	69.1	21.0
Fried beef liver	121	53.3	15.8
Beef liver	131	77.8	22.7
Beef liver	150	58.6	16.1
Beef liver	152	51.2	15.1
Veal liver	70	50.8	13.2
Veal liver	97	69.5	20.2
Veal liver	158	72.1	19.5
Pork liver	122	94.5	29.8
Pork liver	153	60.4	18.7
Pork liver	159	71.4	20.0
Lamb liver	96	59.1	17.2
Beef kidney	81	36.2	8.3
Beef kidney	126	32.1	6.4
Stewed beef kidney	112	14.7	5.2
Pork kidney	62	47.9	10.5
Pork kidney	83	45.4	9.9
Pork kidney	137	45.0	9.1
Stewed beef heart	54	15.9	3.3
Stewed beef heart	87	24.4	7.3
Beef heart	133	42.4	8.2
Beef heart	143	37.0	7.0
Veal heart	155	50.7	10.6
Beef spleen	76	23.3	6.2 [1]
Stewed beef spleen	109	21.1	6.3 [1]
Beef spleen	130	38.8	8.2
Fried beef round	42	34.1	9.1
Beef muscle	105	21.5	6.4
Roast round of beef	107	20.0	4.3
Beef round	128	37.0	8.7
Beef round	145	30.2	7.7
Broiled beef round	147	22.5	...
Fried beef round	148	19.2	...
Beef round	151	33.0	8.2
Beef round	154	27.8	6.9
Beef round	157	27.9	7.8
Fried veal steak	45	28.9	8.4
Veal hindquarter	103	33.0	8.9
Veal hindquarter	129	36.4	9.1
Veal hindquarter	134	31.4	6.8
Lamb leg	80	32.7	8.5
Pork ham	32	20.3	5.6
Fried pork ham	52	14.4	3.7
Pork ham	95	27.0	6.6
Pork ham	124	35.0	8.3
Pork ham	139	33.0	8.4
Smoked ham	47	25.2	8.2
Fried smoked ham	48	30.2	14.3 [1]
Smoked ham	102	19.5	5.6
Smoked ham	116	13.1	3.9
Tender ham	72	14.0	4.4
Tender ham	117	15.3	5.2
Tender ham	119	23.0	7.7
Boiled ham	115	19.3	6.7
Boiled ham	101	18.3	6.0
Pork loin	36	21.2	5.6
Broiled pork loin	37	20.8	4.5
Fried pork loin	38	23.2	5.6
Pork loin	74	21.4	6.1
Pork loin	125	22.2	6.1
Pork loin	156	22.2	6.7
Beef pancreas	64	18.0	5.8
Beef tongue	82	22.0	6.1
Beef brain	132	23.2	5.1
Beef brain	144	22.2	4.7
Beef lung	138	30.4	6.2
Light chicken	68	29.7	7.3
Dark chicken	66	23.8	6.1
Cod muscle	135	13.5	2.3
Salmon muscle	136	26.4	7.4

[1] Calculated approximation.

shown the dark meat to be the more potent vitamin source (Waisman and Elvehjem, '41 b). The muscle of salmon is a richer source of nicotinic acid than cod muscle, the values being 7.4 and 2.3 mg., respectively. Since we were interested in the nicotinic acid content of meat as prepared for consumption, we assayed various samples of home-cooked and commercially-prepared meats. The analysis of fried beef liver, fried beef muscle and fried pork muscle showed very little loss during the cooking process. Assays on stewed beef heart, kidney and spleen showed losses as high as 50% of the vitamin during cooking. The results of analyses of broiled and roasted beef muscle indicate that some nicotinic acid is lost during the cooking process. Commercially-prepared products such as boiled ham, smoked ham and tenderized ham were also analyzed for their nicotinic acid content. All showed some loss due to the processing. The results of these assays are in close agreement with the biological assays.

DISCUSSION

Many of the samples used for this work had been assayed previously by the biological method, and it was of special interest to compare the results obtained by the two methods. The two organ tissues, liver and kidney, found to be the most potent sources of nicotinic acid by biologic assay on the dog were also found to be the most potent source by the chemical method. However, the values obtained from the two assays varied to some extent. The chemical method gave an average value of 18 mg. per 100 gm. of fresh liver tissue, but the biological assay gave a value of 25 mg. Values obtained from the assays of fresh kidney were in similar disagreement. On the other hand, the two methods gave values for muscular tissue and less potent organ tissues that were in close agreement. It appears from such results that growth responses obtained in the biological assays, especially on liver and kidney tissue, are not due entirely to the nicotinic acid content of the meat but to other factors present in the meat which are required by the dog and are not present in sufficient amounts in the basal ration.

The results of our assays have quite closely paralleled those of the other workers or have been somewhat higher. We feel that such variation is due both to methods of extraction and analysis used by the various workers and to the source of meat. It is known that variation in the diet may affect the vitamin content of the tissue.

The loss of some of the vitamin potency in cooking is not likely to be due to the destruction of nicotinic acid since it is a very stable compound. However, being water soluble, it is probably leached from the meat during the processing and remains in the cooking water.

SUMMARY

Nicotinic acid content of meats was determined by the cyanogen bromide-aniline method.

Liver and kidney were the richest sources of nicotinic acid. The average value for liver was 18 mg. per 100 gm. of fresh tissue. All animal tissue contains a significant amount of nicotinic acid.

Cooking and commercial processing caused some loss of the vitamin.

LITERATURE CITED

BANDIER, E. 1939 Quantitative estimation of nicotinic acid in biological material. Biochem. J., vol. 33, p. 1130.

———— 1940 On nicotinic acid—especially methods for its quantitative estimation in organic material. Copenhagen, Einar Munksgaard.

HENDERSON, L. M., H. A. WAISMAN AND C. A. ELVEHJEM 1941 Distribution of pyridoxine (vitamin B_6) in meat and meat products. J. Nutrition, vol. 21, p. 589.

KARRER, P., AND H. KELLER 1939 Bestimmung von nicotinsaureamid in tiereschen organen. Helv. Chim. Acta, vol. 22, p. 1292.

KRINGSTAD, H., AND T. NAESS 1938 Über die kolorimetrische bestimmung von nicotinsaüre und nicotinsaureamid. Naturwissenschaft, vol. 26, p. 709.

KODICEK, E. 1940 Estimation of nicotinic acid in animal tissues, blood and certain foodstuffs. I. Method, II. Applications. Biochem. J., vol. 34, pp. 712, 724.

MELNICK, D., AND H. FIELD, JR. 1940 Determination of nicotinic acid in biological materials by means of photoelectric colorimetry. J. Biol. Chem., vol. 134, p. 1.

MICKELSEN, O., H. A. WAISMAN AND C. A. ELVEHJEM 1939 a The distribution of vitamin B_1 (thiamin) in meat and meat products. J. Nutrition, vol. 17, p. 269.

———— 1939 b The distribution of riboflavin in meat and meat products. J. Nutrition, vol. 18, p. 517.

WAISMAN, H. A., O. MICKELSEN AND C. A. ELVEHJEM 1939 The distribution of the chick antidermatitis factor (pantothenic acid) in meat and meat products. J. Nutrition, vol. 18, p. 247.

WAISMAN, H. A., O. MICKELSEN, J. M. McKIBBIN AND C. A. ELVEHJEM 1940 Nicotinic acid potency of food materials and certan chemical compounds. J. Nutrition, vol. 19, p. 483.

WAISMAN, H. A., AND C. A. ELVEHJEM 1941 a Chemical estimation of nicotinic acid and vitamin B_6. Ind. and Eng. Chem., Anal. Ed., vol. 13, p. 221.

———— 1941 b The vitamin content of meat. Burgess Publishing Co., Minneapolis, Minnesota.

MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1942 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute at Boston on April 1, 1942.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1941 must be in the hands of the Secretary by January 10th, 1942. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

ARTHUR H. SMITH
Wayne University College of Medicine
Detroit, Michigan

SECRETARY, AMERICAN INSTITUTE OF NUTRITION